M* for Origin 6.0
Instruction Manual

Kristian Schilling

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1 General notices and disclaimer

This implementation of MStar is not a standalone executable. It is a macro for the Origin platform consisting of a project file and external script files that are invoked by user interactions on active objects inside the project. The script files extended with `.ogs` are plain ASCII files.

This macro will run only on the 6.0 version of Origin. Service Release 4 *must* have been executed on the Origin installation. SR4 is available from OriginLab's website. You can consult the Help menu to find out whether SR4 has been installed.

The program performs user interacted evaluations of sedimentation/diffusion equilibrium experiments performed on the Beckman Optima XL-A/XL-I analytical ultracentrifuge. The M^* function developed by CREETH and HARDING is calculated, providing model independent evaluation of sedimentation equilibrium. The program works with absorbance and interference data. It thus combines the MSTAR program bundle by HELMUT CÖLFEN.[1] In fact, it is an exact reproduction of that software, following the same procedures and producing equivalent output files. Though minor features have been added, the sole aim in development was to provide a more user-friendly platform and to perform analysis on a whole concentration series.

No warranty whatsoever is given for the correct functioning of the program. Any possible damages to your system arising during operation of this program do not lie in the responsibility of the author.

This program is placed in the public domain and may be distributed freely, however, only as a total package and unmodified. Industrial users are asked for a license fee of EUR 1400 on a voluntary basis, thus appreciating the effort in creating this program and the savings in internal costs in evaluating equilibrium runs. Institutional users are granted free usage.

Please do not remove the Nanolytics logo and the copyright notice on the platform and the printouts (though it is possible). You may alter and customize the script files at your own risk. The source code is not documented and no support requests due to modifications in the script files will be answered. Please also observe the *known bugs and limitations* section.

Other support requests, bug reports and suggestions should be adressed (only e-mail please) to Kristian Schilling, Nanolytics GmbH, Dallgow (Germany), `schilling@nanolytics.de`.

Any feedback on how you get along with the program and where it is applied is appreciated.

[1] as decribed in H. Cölfen, S. Harding, *Eur. Biophys. J.* **25**, 333 (1997). Consult this paper for further references on the M^* function.

2 Getting the stuff

Required files are

`MStar.opj`	Origin project containing the user interface
`equil.ogs`	macro script file
`eq_data.ogs`	macro script file
`main.ogs`	plot template
`results1.otp`	plot template
`results2.otp`	plot template
`dataconc.otw`	worksheet template
`extrapol.otw`	worksheet template
`import.ogs`	import routines
`kstools.ogs`	shared generic routines
`import.otw`	worksheet template
`logimprt.otw`	worksheet template
`params.otw`	worksheet template
`time60.dll`	DLL for reading file time/date stamp
`MStar.pdf`	this documentation in Plain Document Format
`MStar.ps`	this documentation in PostScript Format

The first set of files must be placed in a subdirectory `equil` of the Origin software directory. For version 6.0, this directory is commonly named `Origin60`.

The second set of files goes into a subdirectory named `kstools`. The reason for this lies in the fact that these scripts among others are also used by other macros I wrote and I was too lazy to change the path structures for this public release.

It is recommended to set the read-only flag on the file `MStar.opj`. Do not save the project under this name once you have loaded data and worked with it. Rather save it under another name. You can load it later and continue working with it regardless where it has been saved. All data, cursor positions and variables are saved, you can always continue a session later on and will find it just like you left it.

Additional files provided are sample projects, one for simulated data and one for real data. The real data files for a 10,000 g/mole PEG in water are well suited for testing the J_a extrapolation described in section 3.3.2.

3 Operating M^* for Origin

3.1 Launching and operation basics

To use MStar for Origin, simply launch Origin and load the MStar.opj project located in the `equil` subdirectory of the Origin software directory. You should come up with something like shown in Fig. 1.

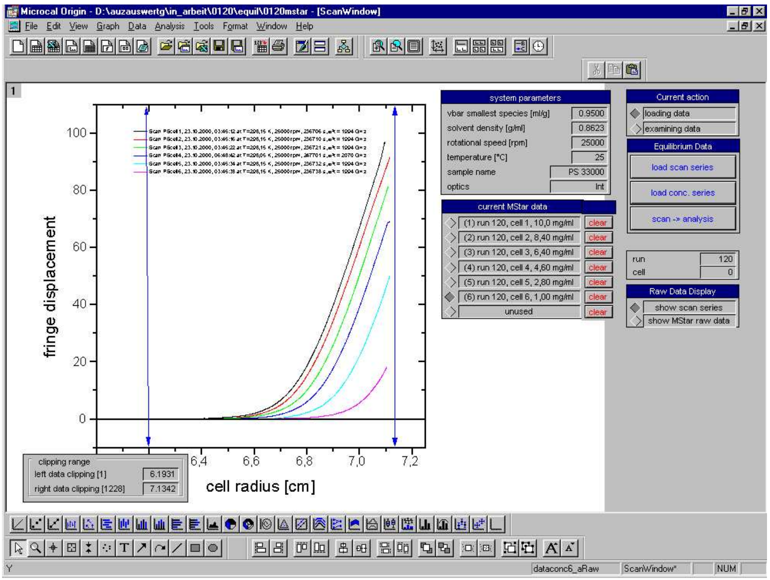

Fig. 1: *Appearance of the MStar.opj project.*

Depending on your screen resolution and Origin desktop configuration, the interface may appear different on your computer. A resolution of 1024 x 768 pixels is absolutely recommended. If you open a lot of toolbars, the working area may become very small as well as labels and buttons.

If you open few or no toolbars at all, the working area becomes very large and some control objects might be placed outside the visible area. Try to find a configuration like on Fig. 1, this should work in all further windows you will encounter.

Except for printing, you should not use the Origin menu at all. All operations are carried out using the control buttons on the plots and other control objects like cursors that will appear inside the plots when needed.

For further tasks, however, you can always address the menu. You have full access to Origin functionality to perform actions not integrated into this project.

If you wish to save your results, you can save the entire project under a new name. If you reload the project, all data and settings will be preserved and you can continue working with these data. You can also create ASCII output of your data in analogy to H. Cölfen's MStar but this data cannot be reloaded.

It is recommended to use a color printer for output since the results for different scans are distinguished by color.

Basically, the project is divided into two major sections as shown in Fig 2: loading data and evaluating data. A third section offers some evaluation plots but performs no further actions on the data.

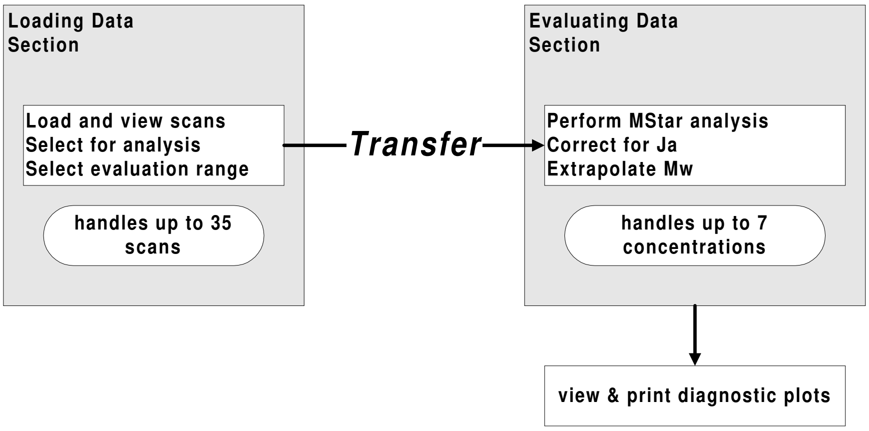

Fig. 2: *Basic project structure.*

The two sections are totally independent. Clearing data in the loading section will not affect any data stored in the evaluation section. In return, clearing evaluated data will not delete imported scans in the loading data section.

The user can switch between the loading data and the evaluating data section anytime. The switch shown in Fig. 3 toggles between loading and evaluating data.

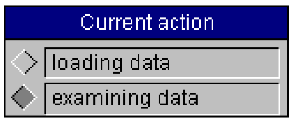

Fig. 3: *Master switch*

Up to 35 scans can be imported[2], then, an appropriate scan can be selected for evaluation. The selected scan is transferred to the evaluation section. After this, other scans can be loaded and transferred. Up to seven scans can be kept in the evaluation section simultaneously. Most likely, these would be scans for different concentrations in different cells.

When all concentrations are evaluated, the user can extrapolate or regress for M_w or A_2, respectively.

[2]in manual mode, using the log mode, an unlimited number of scans can be imported, see below.

3.2 Loading data

In this section, the following can be performed:

- Import a scan series
- Import a concentration series
- Transfer scan or data range to evaluation

The user can decide whether to load a sequence of scans for one cell or a concentration series covering more than one cell. By pressing the corresponding button of the palette shown in Fig. 4, the open file box is invoked.

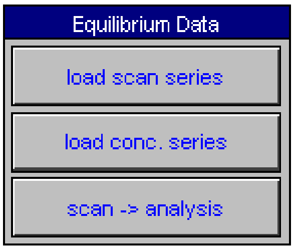

Fig. 4: *Loading commands*

Loading a scan series permits to follow the approach to equilibrium with the progress of time. The following box allows you to preset a cell number and an optical system. From this input, corresponding search masks are generated in subsequent file selection. *Loading a concentration series* is a shortcut most likely applied when the user has already decided to take the latest scans in all cells. These scans need not be located in the same directory.

3.2.1 Loading a scan series

Loading a scan series permits the user to check whether equilibrium has been reached by displaying concentration profiles of one cell with progressing time. The user is prompted for a cell number and the optical system to be used in the `.ip`*n* or `.ra`*n* extension during import, setting a mask for conveniently selecting AUC output files. Use 0 as a cell number for a wildcard. The mouse can be dragged over multiple files to select for import so they need not be selected separately. After selection, the files are transferred into the list box at the bottom of the dialogue by pressing the `add` button. Clicking on `remove` will delete the highlighted file from the list of selected files. When the selection is completed, pressing the `OK` button closes the dialogue, and all files currently listed in the right of the box are imported.

You can also preselect between file select and log select. Loading by log lets you select the Beckman log file where all scan files are listed. Thus, you can load all files in a directory with a single click. You will be allowed e. g. to skip every other scan. This feature is useful for getting an overview over the course of the experiment. It will only work in the original Beckman directory structure. For other limitations refer to section 4.

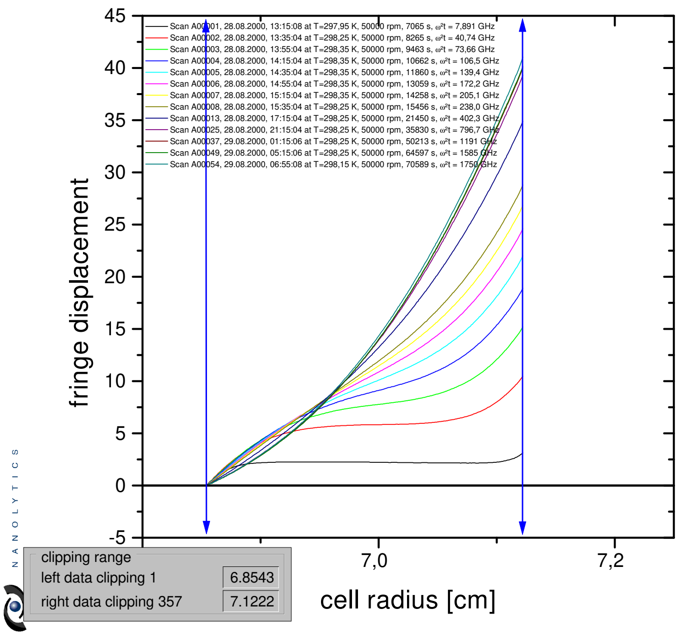

Fig. 5: *Importing a scan series.*

Upon import, the user comment entered in the Beckman data acquisition software for this cell is displayed. The user is prompted for system parameters that are copied from the currently entered values so they can simply be acknowledged if they have not changed since the last import procedure.

Fig 5 shows a typical scan series import. The most important information on the imported scans is summarized into a legend.

3.2.2 Loading a concentration series

If the approach to equilibrium is not of interest because the user is certain that equilibrium has been reached, he might want to import scans from *different* cells covering the whole concentration series. This means that *only one* import procedure is neccessary for loading the entire data needed for evaluation.

For this purpose, use `load concentration series` rather than `load scan series` and select the desired scans as described in the previous section.

If you have used six-channel-centerpieces, load a scan covering multiple channels *only once*. Refer to section 3.2.5 for instructions on how to divide a six-channel-centerpiece scan into sections for evaluation.

3.2.3 Viewing current M* data

The imported data is not automatically available for evaluation. The `loading data` section is intended to be a preview and preselection tool for browsing and selecting the available data. For evaluation, a scan must be explicitely transferred into the evaluation section as described below (section 3.2.5). The scans currently transferred into the evaluation section are listed in a box that is always visible (Fig. 8).

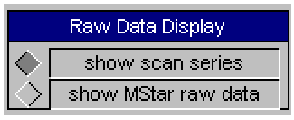

Fig. 6: *Display toggle.*

However, the user might want to view a plot of these data. Toggling the switch displayed in Fig 6 will display a plot of the raw data currently contained in the evaluation section. The datasets are formatted differently to prevent the user from accidentally mistaking these data for the currently *imported* data.

3.2.4 System parameters

Sample name, $\bar{v}$ and other system parameters are displayed in a box shown in Fig. 7. Clicking into one of the data fields will bring up a dialog box where the values can be edited. The system parameters box is also visible in the data evaluation section.

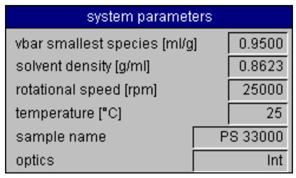

Fig. 7: *Parameters.*

These parameters are used for evaluation, and the program will not log and warn you if parameters are modified during a session. You will have to keep track yourself if you modify, e. g. the particle's $\bar{v}$, for which concentrations the old or the new $\bar{v}$ was used.

The sample name is for information only. The optics entry controls whether a baseline correction (absorbance) or a J_a extrapolation (interference) will be performed.

3.2.5 Transferring scans to evaluation section

Pressing the `scan -> analysis` button in the control palette shown in Fig. 4 lets you select one of the scans from the import section for M^* evaluation. The project provides seven slots in the evaluation section to be occupied simultaneously.

The user is prompted for the slot number he wants to transfer into. The currently transferred scans are listed in a box shown in Fig. 8. Then, the concentration in [mg/ml] is entered and finally the scan selected from a pop-down list. A currently occupied position can be overwritten but the user is warned.

The blue vertical cursors include the data range to be transferred. The current cursor positions are given in an information box in the lower left corner of the desktop. The cursors are moved by clicking and dragging the cursors anywhere but at the tips which would distort them.

You do not need to decide yet which data range you want to evaluate later. This is just to cut off data you will not need in any case so the scaling in the evaluation section will be magnified to the region of interest. This most likely applies for low fillings and for absorbance scans often covering cell radii down to 5.8 cm.

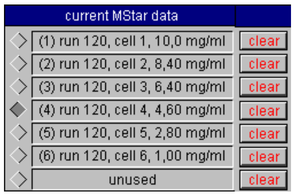

Fig. 8: *Current evaluation data listing.*

Furthermore, you can select one range of a multichannel centerpiece for one evaluation and another range for the next transfer. This is how you divide a multichannel scan into appropriate scans for evaluation of different concentrations.

When handling interference data, the first datapoint in the transfer range is set to zero fringes.

3.3 Preparing data

In principle, you work yourself through the tool palette shown in Fig. 9. Before starting evaluation, you must select the evaluation region using the blue cursors in the data plot. Likewise, meniscus and bottom are set. A baseline correction is possible if you are dealing with absorbance data.

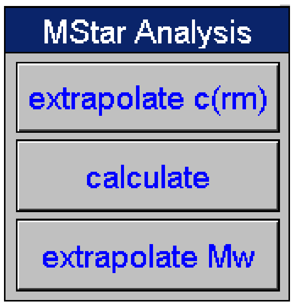

Fig. 9: *Evaluation commands.*

After determining the meniscus concentration, which is a crucial step in the M^* evaluation, the function can be calculated. Eventually, the program will summarize the data you have gathered throughout the concentration series.

The whole procecure runs as follows:

1. Select the scan to be evaluated from the available data listed as displayed in Fig. 8.

2. Set the display mode to A vs. R in the display control panel, depicted in Fig. 10. In all other modes, the additional cursor controls are not visible.

3. Correct for a baseline if dealing with absorbance data.

4. Determine the signal at the meniscus. The procedure varies depending on whether absorbance or interference data is loaded.

5. *After* getting the meniscus concentration, have M^* calculated. A classical evaluation will be performed as well.
6. Extrapolate to the M^* value at the cell bottom; the apparent molar mass is saved for final evaluation.
7. View and enjoy the various diagnostic plots the program offers you.

After the whole concentration series has been evaluated, summarizing plots and calculations yield the final results.

3.3.1 Setting radial positions

When you bring up the A vs. R plot, five cursors are visible inside the plot frame. Use these cursors to set the positions of meniscus, bottom and evaluation limits.

The current positions are reflected in a box in the left upper corner of the layer frame. You can use this box to enter values manually, which will reposition the cursors.

Values inside the data fields may modify as the program rounds input values to the next datapoint.

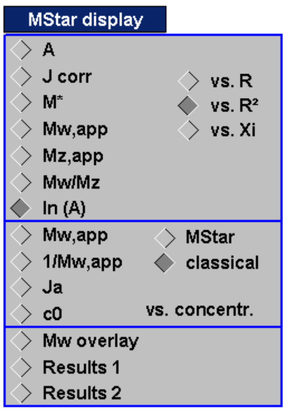

Fig. 10: *M^* display selection.*

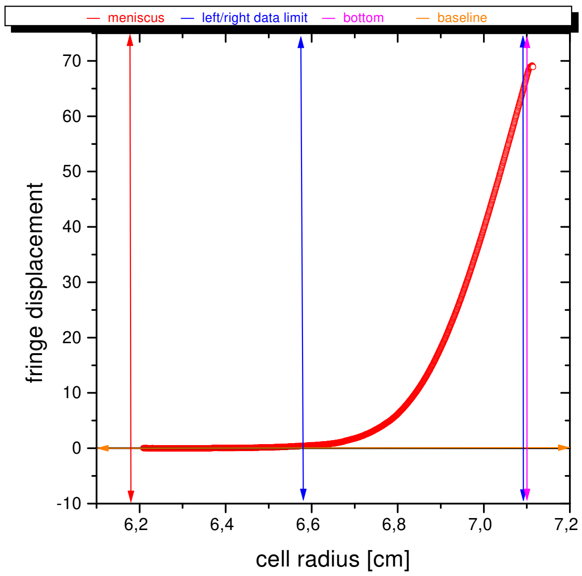

Fig. 11: *Cursors visible in the A vs. R plot.*

Upon any cursor movement, the ξ axis will be recalculated. You can use this as a workaround to update ξ. This may be neccessary when data to be plotted against ξ is not visible. Most likely, the reason would be that the ξ axis got lost somewhere. I'm working on it.

If handling absorbance data, the horizontal cursor can be moved, setting the baseline. This value can, alike the others, be set manually by entering the desired value into the box.

3.3.2 Determining the meniscus concentration

For absorbance data, the solute concentration at the meniscus is derived from a simple linear extrapolation in the meniscus region. For interference data, a more sophisticated procedure is neccessary to obtain the so called J_a, the solute concentration at the meniscus, expressed in fringes. The program decides on what procedure to apply, depending on the selected optics.

Pressing the `extrapolate c(rm)` button in the control shown in Fig. 9 either brings up two vertical cursors (for absorbance data) or launches the J_a determination (for interference data).

Absorbance data

Move the cursors to include a region near the meniscus. Upon every cursor movement, a regression line shows the quality of your extrapolation; the resulting $c(r_m)$ is displayed in a text label. This value is stored automatically. Pressing the same button again (now labeled `extrapolation OK`) hides the cursors.

It is always the value from the latest extrapolation that is stored. As soon as you make an extrapolation, the previous value is overwritten.

Interference data

The procedure applied follows the extrapolation described in H. Cölfen, S. Harding, *Eur. Biophys. J.* **25**, 333 (1997). A linear regression is performed on a plot of

$$\frac{j(r)}{r^2 - r_m^2} \qquad \text{vs.} \qquad \frac{I(r)}{r^2 - r_m^2} \tag{1}$$

where the integral $I(r)$ is

$$I(r) = \int_{r_a}^{r} r\ j(r)\,\mathrm{d}r \tag{2}$$

j is an intensity measured in fringes, r is the cell radius, r_m the meniscus position.

The plot should be linear for lower concentrations, for high concentrations it is bent. Fig. 12 shows a typical plot for a rather high concentration. The two cursors can be moved to select an appropriate section of data. The user should select a linear region near the meniscus, avoiding to include a jittery region that is often observed very close to the meniscus.

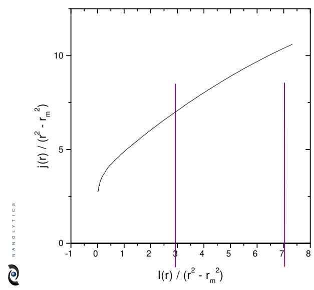

Fig. 12: *J_a extrapolation, step 1.*

Any movement of the cursors provided to accomplish this will launch a regression, the results of which are shown in a text label as well as a box that will pop up.

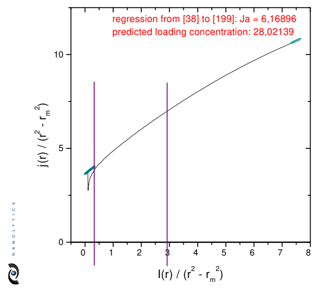

Fig. 13: *J_a extrapolation, step 2.*

J_a is calculated from the regression parameters using the equation

$$J_a = 2 \cdot \frac{intercept}{slope}. \tag{3}$$

Using this value for J_a, the theoretical run of the curve *to the left of the left cursor* and *to the right of the useful data previously selected by the user* is calculated, completing the concentration profile to the full range from the meniscus to the cell bottom. These data are displayed as green triangles as shown in Fig. 13.

As can be seen in Fig 13, the fitted concentration profile near the meniscus might not attach smoothly to the real data at the left cursor. Fig. 14 shows another extrapolation step where the linear region was selected to be much smaller. For this rather strongly bent plot (reflecting interactions), this fit is more appropriate.

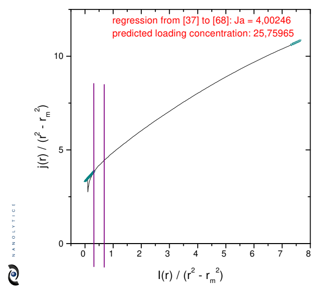

Fig. 14: *J_a extrapolation, step 3.*

The extrapolation towards meniscus and bottom must be performed in two iterations. As the integral in eq. (2) is dependent on J_a (it is an offset that must be added to all values of $j(r)$), the axes are recalculated and the regression is repeated automatically. The user can observe that only in this second iteration, the extrapolated values follow the shape of the curve correctly. This is the only modification to the procedure as described by CÖLFEN and HARDING.

During extrapolation, the user is given additional support by means of two insets. The left inset shows you what the extrapolated data would look like in terms of the original data format (fringes vs. cell radius). Fig 15 gives an enlarged view of this extrapolation. In detail, you see

- the measured data in red,
- the data between the meniscus and your left marker in green,
- the data between the last selected datapoint and the cell bottom in green.

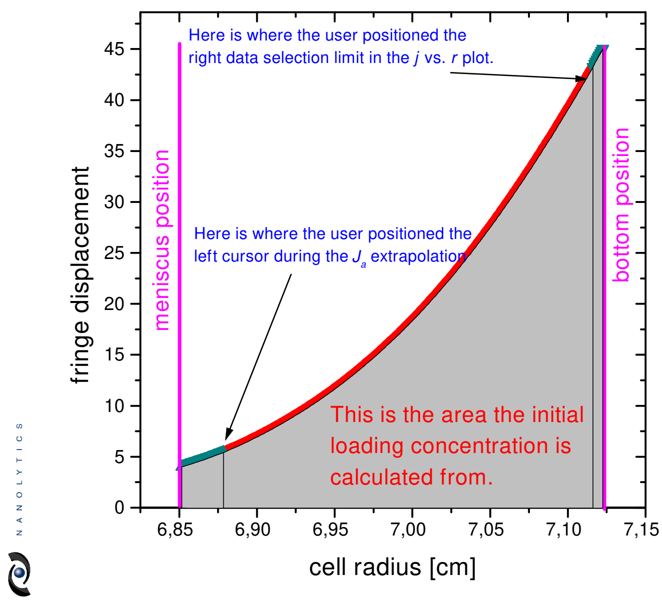

Fig. 15: *Final J_a extrapolation plot and calculation of the predicted loading concentration.*

All original data is shown so you can see whether the extrapolation is well on the measured data in the overlapping region or not.

These data should give a consistent picture. The area beneath this plot is the integral used to calculate the initial loading concentration. This takes into account that

- the data near the meniscus is often jittery and the user's left mark is a good criteria for the limit where extrapolated data is more reliable,

- the data near the bottom is often not resolved, and the user's right evaluation mark is a good criteria for this limit,
- the J_a contribution to the initial loading concentration must be taken into account.

The extrapolated data is used only for calculation of the initial loading concentration. After this procedure, the data is discarded. *For the later calculation of M^*, the original data is used.*

The predicted loading concentration can be used to verify the calculation from a mass balance

- either validating the fringe displacement with a synthetic boundary run
- or by comparing the refractive index increment determined from the J_a extrapolation to a literature value (ref. to section 3.5.2).

The second inset plots all initial loading concentrations determined so far as a function of concentration. This should be a linear plot.

Both plots can also be accessed in full view later (section 3.5.2), but it has proved convenient to have them at hand during the extrapolation procedure.

Important hints for the J_a extrapolation

Note that the neccessary recalculation of the axes, using the currently extrapolated value of J_a, means that the data plotted is more and more modified from the original data with each iteration. For this reason, the cursors should not be moved too often; if many iterations have already been performed, it might be a good idea to quit the procedure, accepting the last extrapolation, and then to start over again. Then, the cursors will be found in their previous positions, and only one movement is required to perform the extrapolation.

In order to minimize cursor movements (and thus calculations), the cursors can be crossed over; it is not neccessary that the left cursor remains on the left.

3.4 Evaluating data

3.4.1 M^* calculation

Upon pressing the button labeled `calculate` in the toolbar shown in Fig. 9, the M^* function is calculated for the active dataset, selected in the box depicted in Fig. 8. Calculation is carried out according to

$$M^*(r) = \frac{c(r) - c_m}{kc_m\,(r^2 - r_m^2) + 2k\int_{r_m}^{r} r\ [c(r) - c_m]\ \mathrm{d}r}\,, \tag{4}$$

where r is the radial axis, $c(r)$ the concentration (measured as fringes, OD or whatever), c_m the concentration at the meniscus, r_m the meniscus position. *c_m is **not** the baseline absorbance!* For absorbance experiments, it is the absorbance at the meniscus, for interference data it is J_a, the determination of which was discussed in section 3.3.2[3]. A baseline is always zero for interference data; for absorbance data, a baseline must be subtracted *prior* to applying eq. 4, which is done automatically.

The constant k stands for

$$k = \frac{(1 - \bar{v}\ \varrho)\cdot\omega^2}{2RT}\,. \tag{5}$$

When the calculation is finished, the program automatically brings up the M^* vs. ξ plot, where an extrapolation of M^* towards the cell bottom can be performed. The value of M^* at the cell bottom ($\xi = 1$) yields the apparent molecular mass for this concentration:

$$M^*(r = r_b) = M_{w,app} \tag{6}$$

The extrapolation is started by pressing `extrapolate M*` in the toolbar from Fig. 9. This brings up two cursors for selection of the linear regression range. Upon any cursor movement, a regression is performed, and the value of M^* at the cell bottom is calculated and diplayed.

This value is stored in the result summary without explicit request. *It will overwrite any recent value stored here.*

3.4.2 Savitzky-Golay smoothing

In addition to the calculation of M^*, the program automatically performs a classical evaluation in the $\ln A$ vs. r^2 plot that should ideally give a line, the slope of which yields $M_{w,app}$.

For this purpose, a Savitzky-Golay regression (called *sliding strip* in H. Cölfen's MStar) is performed on the data. This is a means of data smoothing, where a number of points to the left and to the right of the processed

[3]Of course, J_a is an offset that is not only added to the meniscus concentration, but to all datapoints.

datapoint are used for a regression. From the regression, a smoothed value for the datapoint in question is calculated.

The user can select the order of the regression polynome from first to fifth order in the Savitzky-Golay options, Fig. 16. The user can also modify the strip length. A default value is calculated from the number of datapoints inside the selected data region. You can use this value or an arbitrary value that is entered by clicking onto the options. The current values for both settings are displayed in parantheses.

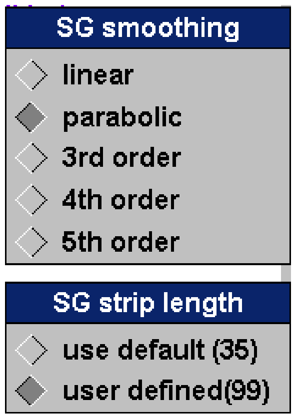

Fig. 16: *Smoothing option.*

From the local slope at each datapoint k, a value for the apparent molecular mass is calculated from the classical relationship

$$M_k(r) = \frac{2\,R\,T}{(1-\bar{v}\,\varrho)\,\omega^2} \cdot \frac{\mathrm{d}\,(\ln c_k)}{\mathrm{d}\,(r_k^2)} \qquad (7)$$

The $M_{w,app}(r)$ datasets are stored and can be viewed. Ideally, these datasets should give a constant.

3.5 Viewing results

Fig. 17 shows the palette where the user can select the plots to be viewed. Any click on the radio buttons will cause the plot to be refreshed, using the desired axes. Some of the plots are for a single dataset, in that case, the active dataset selected in the box shown in Fig. 8 is used. Other plots summarize results from the entire concentration series.

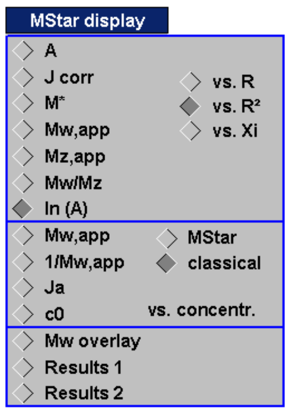

Fig. 17: *Plot selection.*

For single dataset plots, the user can select whether to plot on r, r^2 or ξ. For linear and reciprocal plots of $M_{w,app}$ vs. concentration, you can choose between the M^* and classical evaluations.

There are also two plots that are intended as printouts.

In all plots, only the data range selected by the user is displayed. This range is selected

- in single dataset plots, by the cursor settings in the A vs. R plot,
- in concentration series plots by the settings in the box shown in Fig. 18.

3.5.1 Results for one concentration

Plot of A

This plot displays the raw data as absorbance or fringe displacement.

Plot of J_{corr}

This plot shows the data actually used for calculation, including the baseline and J_a corrections. For interference data, also the extrapolated data are displayed to judge the goodness of the J_a extrapolation procedure. Refer to fig. 15.

Plot of M^*

This plot displays the M^* function. This plot must be selected for an extrapolation of M^* towards the cell bottom. It is automatically displayed when the user launches this extrapolation by pressing `extrapolate Mw` in the toolbar shown in Fig. 9. It also comes up after calculation of the M^* function when the user has pressed `calculate` in that toolbar.

Plot of $M_{w,\,app}$

This plot brings up the local values for $M_{w,\,app}$ calculated from the local slopes in the classical $\ln A$ vs. R^2 plot. It should be a constant for a non-interacting system.

To calculate the average, click the `extrapolate M(w)` button. The data between the cursors will be averaged, the average is shown in a text label and a horizontal line.

Plot of $M_{z,\,app}$

This plot brings up the local values for $M_{z,\,app}$ calculated from the local slopes in the classical $\ln A$ vs. R^2 plot. It should be a constant for a non-interacting system.

$M_{z,\,app}$ is calculated according to the following equation:

$$M_{z,\,app}(r) = M_{w,\,app}(r) \;+\; \frac{1}{k} \cdot \frac{\mathrm{d}}{\mathrm{d}(r^2)} \left[\ln \left(\frac{1}{r} \, \frac{\mathrm{d}A(r)}{\mathrm{d}r} \right) \right] \tag{8}$$

where the experimental constant k has the same meaning as in eq. 5.

We stress here that uv-absorption data are usually of insufficient quality to obtain realistic estimates of $M_{z,\,app}(r)$ since a double derivative is required. This is more suited to interference optics.

Plot of $\ln A$

This plot shows the classical equilibrium evaluation according to eq. 7.

If the data is plotted as a function of r^2, the Savitzky-Golay regression will also be displayed as a blue line, smoothing the data to some extent. The missing datapoints at the left and the right of the smoothed data are due to the fact that the sliding strips need a number of excess datapoints at the ends, dependent on the strip length.

3.5.2 Results for concentration series

Plot of $M_{w,\,app}$ vs. concentration

This plot displays the apparent molecular masses, derived from extrapolation of M^* towards the cell bottom, as a function of concentration. This applies for the selection `MStar` in the check box on the right. Choosing `classical` will plot the classically obtained mean values for M_w (eq. 7).

A linear regression is automatically performed; the resulting estimate for M_w at infinite dilution is shown in a text label.

If the regression line is not visible or the plot is badly scaled, a second click on the display option might cure the problem. You might also check whether any extrapolated values exist at this point.

Plot of $1/M_{w,\,app}$ vs. concentration

This is the reciprocal analogue to the previously described plot.

In addition, the slope is evaluated to yield the Second Virial Coefficient A_2 that is also displayed in the text label.

J_a / baseline vs. concentration

This plot is intended to be a check for correct J_a extrapolation. It also works for absorbance data. With increasing concentration, an increasing J_a is expected. If the molecular masses do not scale in the expected way, this plot might reveal that one or several estimates for J_a clearly deviate from a generally observed trend.

Though these data are not expected to scale linear, a regression is carried out, yielding the y axis intercept to check whether J_a for inifinite dilution is reasonably close to zero.

Plot of J_0/A_0 vs. concentration

The initial loading concentrations of all evaluations are stored and can be viewed in a J_0 vs. c plot when all evaluations are complete. These should scale linear with concentration. An erranous extrapolation for J_a will reflect in a bad value for J_0 that may be identified in this plot. In this case, repeat the J_a extrapolation for this scan.

For interference data, the initial loading concentration as measured in fringes can be converted into a concentration in [mg/ml] using the relationship

$$\mathrm{d}c = \mathrm{d}\phi \cdot \frac{\lambda}{l \cdot \frac{\mathrm{d}n}{\mathrm{d}c}} \tag{9}$$

where $\mathrm{d}\phi$ is the fringe displacement, and λ the laser wavelength of 675 nm.

As the sample concentrations have been entered by the user, the refractive index increment can be calculated from the slope of this plot. A text label displays this value for comparison to the expectations. The y intercept is also displayed and should be close to zero.

For absorbance data, the extinction coefficient is calculated instead of the refractive index increment. In both cases, an optical pathlength l of 12 mm is assumed.

M_w overlay

This plot displays the local weight average molecular masses as calculated from eq. 7 from all concentrations as a function of fringe or absorbance units. The data should form a master curve that can be manually extrapolated to $c = 0$.

3.5.3 Summarizing plots

Results 1

Most of the plots discussed above are summarized on this page. These are all plots showing evaluated *datapoints* as functions of R or ξ. The master curves of M_w and M_z vs. absorbance or fringe displacement, respectively, are also given. Plots using the sample concentration as the x axis are compiled in the plot **Results 2**.

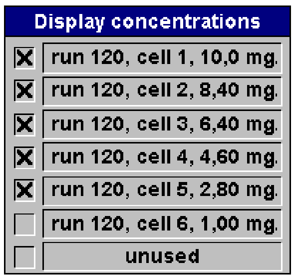

Fig. 18: *Result selection toolbar.*

There are three control elements on this page.

The palette shown in Fig. 18 allows the user to exclude single datasets from display and evaluation. All concentrations that are not checked will not only be excluded from display on this plot; they will also not occur in any regressions performed on the final results.

This selection can also be made on the page **results2**. On the original desktop, the selection of datasets is performed by checking boxes next to the list of available datasets, refer to fig. 8. The check boxes will appear whenever a plot vs. concentration is selected.

Whereever you select or deselect datasets - this selection will affect any other page as well. If you have the impression that data you evaluated is missing, check with this palette.

The second control element allows you to select whether or not the M_z results should be displayed. These data are mostly useless, and the overview is more legible when only six plots are displayed.

In addition, the results page offers the option to conveniently scale all molecular mass axes in a single step. Pressing the button `set M axis range` in the palette depicted in Fig. 19 brings up a dialogue where the user can enter a display range that will apply for all M axes.

The `update display` button should be pressed after the selection of datasets (Fig. 18) has been modified. It can also be used after entering the page, in case the display is messed up in any way, what sometimes occurs for unknown reasons.

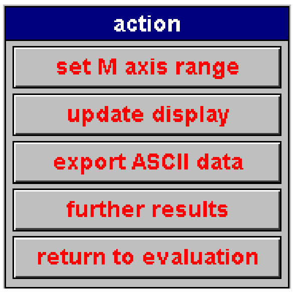

Fig. 19: *Results toolbar.*

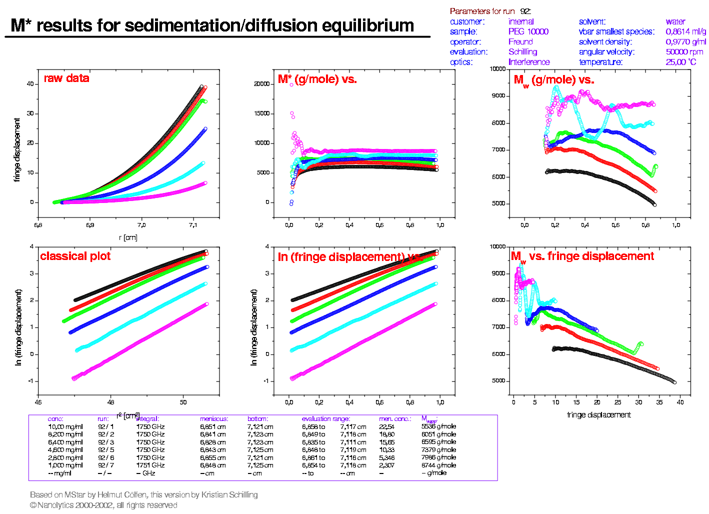

Fig. 20: *Results overview, page 1.*

All calculated distributions are exported as ASCII files when you press the button `export ASCII data`. You will be prompted for a file name. The 7 available positions for evaluation are enumerated into the file name. Extensions will be automatically appended following the conventions given by H. Cölfen in the original MSTAR program. The file format and the scope of exported results is also designed to reproduce files created by the original version.

This routine is only implemented for reasons of compatibility. You should not use this option to save your results, rather save the entire project. Exported files cannot be reimported. There is no possibility to recalculate or modify your results if the project itself is not stored.

`Further Results` brings you directly to the page **Results 2**; `return to evaluation` to the standard interface.

Upon the first display, all data are scaled to show all data in the individual plot. This might mean that plots might be inconveniently scaled due to single datapoints that are way out of range. Use the `set M axis range` option to uniformly scale the M axes. Data outside the layer frame will be clipped.

You can scale other axes, print out and add additional text labels using normal Origin functionality from the menu.

Results 2

This page summarizes all data on the entire concentration series. Plots of $M_{w,app}$ versus concentration are included in linear and reciprocal scales, the plots of J_a and J_0 (or absorbance, respectively) are given to check the meniscus concentration extrapolations.

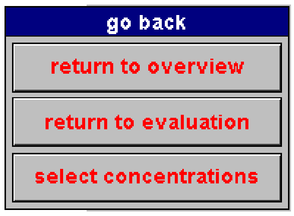

Fig. 21: *Results 2 commands.*

The buttons shown in Fig. 21 bring the user to the first results page or back to the standard interface. In addition, the user can include and exclude datasets from display and regression in analogy to the selection palette on the **Results 1** page, Fig. 18. Any changes will be reflected on the **Results 1** page and in the evaluation section.

Finally, the user can select whether to use the M^* or classical $M_{w,app}$ values for display.

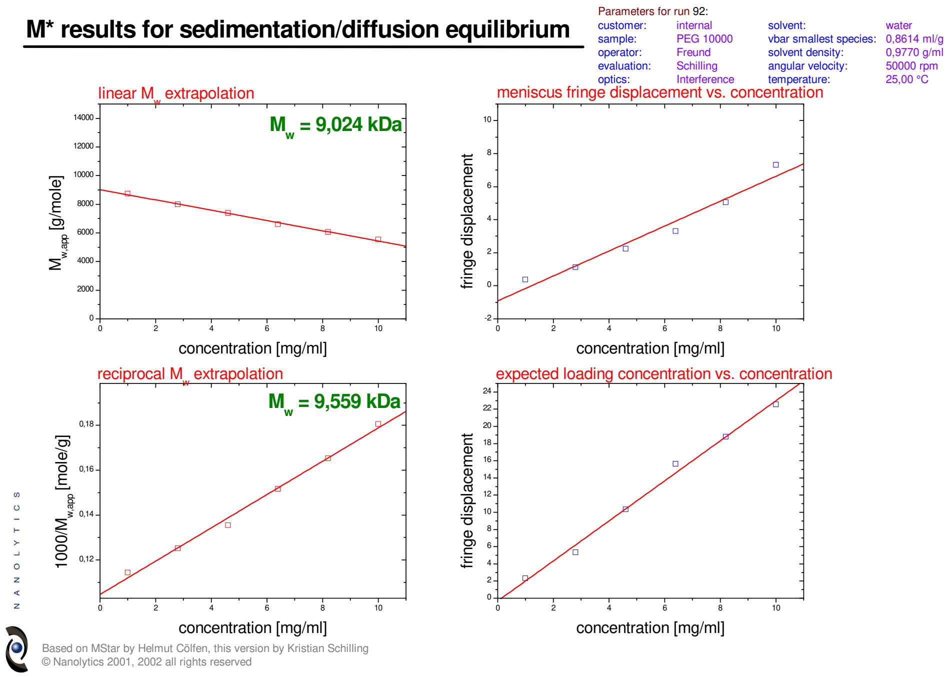

Fig. 22: *Results overview, page 2.*

4 Known bugs and limitations

In some cases, data displayed in one or other layer may be hidden, this may also happen with the entire layer in question (that is, also axes and labels). Double clicking the layer 1 icon in the left top corner of the plot and just confirming with `OK` will mostly cure this problem.

Though an Apple version of Origin does exist, it has never been tested whether this macro will execute on a MacIntosh platform as well. For sure, running the DLL (compiled for Windows) will cause an error. I have no idea if other problems will arise and I cannot provide any support for Apple computers.

But there might be problems on Windows computers as well. This macro has been tested on Win98 and Win2000, but apparently there can be problems on one computer that do not arise on another. This must have something to do with Origin itself. I have never yet been able to find out a possible explanation. If you want to use this macro, just try it out - if it won't behave, you might try it on another computer.

Two issues concern file import. Importing by log reads the Beckman log file that can have different formats, namely, a number of lines to be skipped varies depending on your version of the data acquisition software. This only applies when you want to import all scans on a cell, so the problem is not crucial. You can try, however, to modify the line `skipfactor=4;` in the `import.ogs` script; try a value 3 instead.

The other is about single file select: in principle, you can select multiple scans and transfer them alltogether with the `add file` command. Too bad Windows makes some mess out of the file order - they will be mixed randomly and this is the order they will be loaded. You can either transfer them into the evaluation slots restoring a sensible order or double click them one by one in the file selection box which will append them to the selection list in the order you transferred them.

For reasons not understood, the provided `timdll32` runtime library, needed to retrieve the time stamp of the data file, will always output a time two hours too early. I could have this corrected after `timdll32` execution, but consider this a nice gesture by the system and it can't hurt if your boss thinks you were busy somewhat ealier.

I hope that Microcal will soon provide an internal function to accomplish this task making the DLL obsolete so it's not really worthwhile to repair it.

If you have any problems such that the script, or sections of it, do not appear to execute, please check first whether your version of Origin 6.0 has been patched with the Service Release 4. This is essential. You can check in the Help... About Menu.