

UltraScan Flowchart for the Analysis of Sedimentation Velocity Data

Step 1: For velocity data recorded in intensity mode, convert the SV intensity data into pseudo-absorbance data:

1. *Utilities: Convert Intensity to Pseudo-Absorbance.*
2. *Select Directory* with intensity data.
3. Select the the reference channel containing water only by clicking on the appropriate cell under “Please Select a Cell”. Then click on *Show Channel 1* or *Show Channel 2*.
4. Click on *Mark this Channel as Reference*.
5. Draw with the mouse cursor a rectangle over a smooth area (without spikes) to average data points in between.
6. For each cell, click on *Show Channel 1*, *Show Channel 2*, *Convert This Cell*.
7. The pseudo-absorbance SV data is now saved in two subdirectories designated channel-1 and channel-2, and each channel needs to be uploaded (see below) as a separate run. Reference channels (usually one) can be deleted before uploading the SV pseudo-absorbance data to the USLIMS database.

Step 2: Upload experimental data from XL-A/I to database and associate it with peptide/nucleic acid sequence(s) and buffer composition data:

1. Database: Commit Raw Experimental Data to DB.
2. Load Run in the Experimental Data Table window.
3. Specify the experiment, i.e. optics and type, using the check boxes (*Absorbance*, *Velocity*, etc.).
4. *Select Data Directory* (this is the data directory where the Beckman Data Acquisition stores the experimental data from the XL-A/I) and select the appropriate data folder.
5. Inspect the run details and diagnostics in the *Details for Velocity Absorbance Data* window. Also enter a “Run Identification” and click on *Accept*.
6. Go to *Sedimentation Data Entry* window and click on *Review Dataset*.
7. Review all cells using the *Next Cell* button and pick centerpiece and rotor for each.
8. *Commit to DB* and click OK to close *Sedimentation Data Entry* window.
9. *Select Investigator* in the *Experimental Data Table* window.
10. Enter a verbose description of experiment.
11. Activate the drop-down list with cell information and click on the first cell.
12. In the dialog that pops up, select a buffer, and 1-3 peptide and/or nucleic acid sequences for each channel in the experiment. For each cell, click *Add DB Entry* and *Close* before going on to the next cell from the drop-down list.
13. *Add DB Entry* in the *Experimental Data Table* window to commit the entire run to database.

Step 3: Retrieve raw data from database for editing:

1. *Database: Retrieve Raw Experimental Data from DB: Investigator* (or another selection method).
2. Enter last name of investigator (or *Check Database for Investigator Record*), display the data loaded, and double click an item in the list.
3. Click *Retrieve* and close the *us_db_rtv_investigator* window that pops up after retrieval.

Step 4: Edit experimental data (see [UltraScan Manual](#) for details).

Step 5: Commit edited data to database. This edited data is now a binary dataset and considered to be “Result data”.

1. Database: Manage Results and Analysis Data: Velocity Data.
2. Load Data from Hard Drive.
3. Select the result file (*.us.v) and click on *Backup Result to Database*.

Step 6: Find the limits of the s-value range. For absorbance data, use *Velocity: Enhanced van Holde – Weischet*, and add +/- 20% to the upper and lower s-values of the s-distribution. For intensity data with a lot of time invariant noise, *Time Derivative* is better (smooth data a bit, clip data that has not cleared meniscus, and delete scans that don't exhibit a stable upper plateau. Click on *x: S* to display in s-conversion, and then select *Avg. dC/dt Plot* to plot the dc/dt s-value distribution.

Step 7: Submit a 2DSA analysis request in USLIMS. Locate the dataset and set the s-value limits to values obtained in step 6. Set the resolution for s and f/f0 to the default value 10, and set f/f0 limits to 1-4 or adjust the upper limit based on prior knowledge of the sample (in case of DNA for example). **Turn on time invariant noise, but set Monte Carlo to zero iterations.** Submit to cluster of choice. Optional: For best results also fit the meniscus over 0.03 cm with 10 points. Fit the RMSD values for each meniscus position with the meniscus fitted (*Utilities:Fit Meniscus from 2DSA Velocity Data*). After updating the meniscus in *Utilities, Update Meniscus for Velocity Run*, you can rerun the 2DSA analysis without meniscus fitting, but turn on both TI and RI noise fitting. You will receive 3 files in your e-mail attachment, 2 of which will be the noise vectors.

Step 8: Retrieve e-mail attachments, 2 files: Model file and ti_noise file (with best RMSD if fitting meniscus, fit meniscus, set derivative to zero to determine best meniscus radius). *Load Data* into *Velocity: FE Model Viewer (non-interacting)*, then *Load Model*. Load time invariant noise as well. *Simulate Model*. Click on *Residuals* in *Enhanced Plotting Controls*. *Write Ti/Ri Noise to File*, then *Close*. Click on *3D Plot*. Adjust as needed. Go back to main window underneath entitled *Compare Experimental Data to Sums of Finite Element Solutions*. Click *Save* and *Close*.

Step 9: Go to *Utilities: RI/TI Noise Subtraction from Velocity Run*. Keep run name the same and confirm (*Accept*).

Step 10: Update the DB by replacing the noisy result data (*Database: Manage Results and Analysis Data: Velocity Data*). *Load Data from Hard Drive*, select the noise corrected data from HD and click *Backup Result to Database*.

Step 11: (Optional) Refit 2DSA with corrected meniscus and ti noise correction turned on to get better TI noise. **NOTE: The noisy data may still be loaded in the queue, so make sure to reselect the dataset from the USLIMS list-box and to reselect the cell over to make sure the noise corrected data is used.**

Step 12: Perform 2DSA Monte Carlo analysis without ti noise correction turned on. 50 iterations is a good start. Use the same limits as before.

Step 13: If the 2DSA distribution appears to be a sparse solute situation, and not a smooth continuous distribution of many species, you can further refine the data with a parsimonious regularization using the GA analysis. Use *Initialize GA with 2DSA Distribution*. *Load distribution* (this also works for GA distribution data) and use *Autoassign Solute Bins* and *Save* the data to disk (file: <run_id>.gadistro.dat). Upload this file into the GA analysis on the USLIMS to initialize s and f/f0 buckets.

Step 14: Perform Monte Carlo GA analysis on USLIMS by repeating step 13 with the GA distribution obtained in step 13.