UltraScan-III ver. 2.0 Flowchart for the Analysis of Sedimentation Velocity Data

Step 1: Convert Beckman legacy data into UltraScan-III OpenAUC format

1. *Utilities:Convert Legacy Data*
2. Confirm Investigator setting and local/database selection
3. *Import Legacy Data from HD* with intensity data.
4. Edit Run Information, *Select Lab/Rotor/Calibration*
5. Enter a L*abel* (verbose description for the run)
6. Select the corresponding project by clicking on the *Project* button
7. Confirm experiment type and optical detection system
8. Enter any comments, if applicable
9. Select instrument and operator
10. Click *Accept*.
11. Edit the *Description* field if necessary
12. Navigate to the first channel and select the centerpiece type.
13. Select the proper solution, make sure that the solution contains at least one analyte and a buffer
14. If you have more than one triple, you can click *Apply to All* but verify centerpiece and solution for each triple. Also, check the *Description* field again to make sure the appropriate information is saved.
15. If data were collected in intensity mode, you will need to *Define Reference Scans* by selecting a short region from the air-to-air interface portion of the data.
16. Failed triples or empty triples can be excluded from the run by clicking on *Drop Current Triple*.
17. For equilibrium data from 6-channel centerpieces you should separate each channel with the *Process Subsets* function.
18. When everything has been set you can *Save* the scans to database or disk.

Step 2: Edit experimental data (see UltraScan Manual for details).

Step 3: Perform a Time Derivative analysis and find the limits of the s-value range.

1. Select *Velocity:Time Derivative* and *Load Experiment*
2. Set *Data Smoothing* to ~ 10
3. Set the *Boundary Pos (%)* to zero
4. Delete scans that don not exhibit a stable upper plateau.
5. Select *Average S* to plot the dc/dt S-value distribution (default setting).
6. The correct S limits to choose are the left and right limits of the S-value distribution where all signal returns to baseline. The minimum S-value allowed is 0.2 S.

Step 4: Submit a 2DSA analysis request in USLIMS.

1. Locate the dataset and set the s-value limits to values obtained in step 3, 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).
2. Set the resolution for *S* and f/f_0 to the desired value. 60 is the default for both *S* and f/f_0, the resolution is the number of points into which this variable will be discretized. For

example, if the range is selected from 1-5, and the resolution is set to 40, there will be 10 grid points/S-value, and the increment would be 0.1 S.

3. Set the number of grids to the product of the two resolutions (S and f/f_0) raised to the 0.25 power. Use the next higher integer value if a fraction is obtained. For example, if you have a resolution of 90 for S and a resolution of 60 for f/f_0, you would set the number of grids to 9, because $(90 \times 60)^{0.25}$. = 8.57
4. Turn on time invariant noise and leave all other settings at default values.
5. Submit the job to your desired cluster or calculate locally using the 2DSA GUI method
6. Check queue viewer for job completion
7. Confirm results with *Velocity:FE Model Viewer*, load newly generated time invariant noise file. If random residuals are obtained, proceed with step 5, but if residuals do not look random, and a strong diagonal line in the residual bitmap is apparent, investigate range settings for S and f/f_0 settings and repeat 2DSA with improved ranges.

Step 5: Perform Meniscus fit.

1. repeat submission by also fitting the meniscus over 0.03 cm with 10 points, turning on both time invariant and radially invariant noise. Make sure to load the previously generated time invariant noise when loading the dataset. Use the same range settings as in Step 4.
2. Check queue or e-mail and once results are available, load *Utilities:Fit Meniscus*. Click on *Scan Database* if using the database, and check the *Status* line for new results. Go to *File:Load* to load the desired meniscus fit (check the UltraScan manual for details).
3. After updating the meniscus, confirm the deletion of the scans that resulted in non-optimal RMSD values.

Step 6: Perform a final 2DSA refinement

1. Refine the analysis by re-fitting the data with the improved meniscus value by repeating the analysis with identical range settings as before fitted in Step 5, *except* this time selecting the time- and radially invariant noise that was generated in Step 5, and also *do not* select meniscus fitting. Instead, select *Iterative Refinement* and set the refinement level to 10 iterations. Also refit time and radially invariant noise.
2. Visualize the final results in *Velocity:FE Model Viewer* and save results to database.
3. All subsequent analyses methods should now be based on the model generated in this final 2DSA refinement step.

At this point, multiple analysis options exist depending on the properties of the analyte distribution. If a paucidisperse solution is obtained, parsimonious regularization with the genetic algorithm method is appropriate. Otherwise, the data should be analyzed only by the 2DSA analysis in conjunction with a 50-iteration Monte Carlo analysis. Both options are explained below.

Step 7: Genetic Algorithm analysis.

1. If the refined 2DSA data are appropriate for genetic algorithm analysis, select *Velocity:Initialize Genetic Algorithm* and load the model from Step 6 into the initialization program. See the corresponding UltraScan Manual section for details.
2. Assign initialization and save to disk

3. Log into USLIMS and submit data to Genetic Algorithm analysis. Make sure to select time and radially invariant noise generated in Step 6, but *do not* refit time and radially invariant noise.
4. Select the gadistro file from the UltraScan/results/run-id directory for the correct triple.
5. Visualize results by using the *Velocity:FE Model Viewer*

Step 8: Perform 2DSA Monte Carlo analysis.

1. Use the same limits as before, but *do not* refit time or radially invariant noise, instead, load the noise corrections from Step 6. Select 50 Monte Carlo iterations.
2. 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.
3. When using 2DSA Monte Carlo distributions for the GA initialization, make sure to use the manual GA initalization method in *Velocity:Initialize Genetic Algorithm*.
4. Proceed as described in Step 7.

Step 9: Perform Monte Carlo GA analysis

1. Using the results from Step 7, initialize the genetic algorithm – Monte Carlo analysis as described in Step 7.
2. In USLIMS, load the data, using the noise files generated in Step 6.
3. Select 50 Monte Carlo iterations
4. select parallel processing with 8 program groups.
5. repeat Step 7 by loading the gadistro file generated from the GA distribution model.
6. Submit to desired cluster and visualize results by using the *Velocity:FE Model Viewer*

Step 10: Perform van Holde – Weischet analysis

1. Open *Velocity:Enhanced van Holde - Weischet*
2. Load the desired experiment, applying the noise files from Step 6 (the latest model).
3. Check *Plateaus from 2DSA* and *Use Enhanced vHW*
4. Adjust *Beck Diffusion Tolerance, Divisions, Data Smoothing, % of Boundary,* and *Boundary Position* to desired values.
5. If appropriate, delete early scans to improve resolution and reduce noise. Only keep scans and boundary portions that contribute to well correlated line fits in the linear extrapolations.
6. Select groups, if appropriate, to generate weight averaged s-values for discrete species
7. Display *Distribution Plot* and histogram.
8. *Save Data* and distributions
9. Refer to the van Holde – Weischet manual page for additional details.

Step 11: Overlay combined distributions

1. All van Holde – Weischet distributions and finite element models can be combined into a single plot for easy comparison.
2. Use *Velocity:Combine Distribution Plots (vHW)* for van Holde – Weischet plots
3. Use *Velocity:Combine discrete Distributions* for all finite element models (2DSA, GA, Monte Carlo)