DIY in Lunatic and Stunner with the Homebrew toolkit

Introduction

Quantifying biologics often requires many kinds of measurements and a lot of tedious downstream data processing. That can mean time-consuming, hands-on work with different instruments and software before you can get the results you need. Lunatic (Figure 1A) makes protein quantification easy with a wide dynamic range that can measure concentrations from 0.02–200 mg/mL so you can skip the manual dilutions. Maximize your throughput with high-speed UV/Vis spectral analysis with just 2 µL of sample and up to 96 samples at a time. The unique microfluidic circuits molded into Lunatic plates ensure that there's no cross-contamination or evaporation. Pipet your samples right into Lunatic plates, or hook them up to your favorite automation system if you need higher throughput. Tack on 21 CFR Part 11 compliance if you need it.

Stunner (Figure 1B) takes your protein characterization to the next level by combining Lunatic's high-speed UV/Vis spectral analysis with Dynamic Light Scattering (DLS). Using micro-volume Stunner plates, Stunner measures both the concentration and quality of 96 samples in just 1 hour, and gives you control over how to sync up measurements of sample quantity and quality.

Lunatic and Stunner both collect the entire UV/Vis absorbance spectrum for every sample in every experiment and determine protein concentrations from the absorbance at 280 nm. The Homebrew toolkit lets you put all that absorbance data to work by allowing you to accurately quantify your molecules and, at the same time, create custom outputs and specify the background correction method. Homebrew lets you streamline your workflow by giving you the tools to build custom applications to meet your needs. You can measure the UV/Vis absorbance spectrum, apply your preferred background correction, determine your protein concentration, and calculate statistics on your replicates, all from a single unified interface.

A

B

Figure 1: Lunatic: The next-gen UV/Vis reader (**A**). Stunner: a unique combination of simultaneous quantification and sizing (**B**).

With Homebrew on Stunner, you can add DLS measurements to your outputs along with the UV/Vis absorbance data. DLS measures the changes in light scattering of particles in a solution over time to quantify particle diffusion. If the viscosity of the solution is known, DLS can determine the size and polydispersity of the particles to detect protein aggregates. If the particle size is known, the same methods can be used to determine the viscosity of a solution from the measured diffusion coefficient of the particles.[1] Homebrew on Stunner can determine the viscosity of buffers or high-concentration antibody formulations with a single, easy-to-use platform.

This application note describes how you can use Homebrew to design custom applications in Lunatic and Stunner, such as instant statistical analysis of your results and determination of the

viscosity of a solution using NIST bead standards. These examples barely scratch the surface of what is possible with this powerful toolkit.

Homebrew on Lunatic

Statistics at your fingertips

Replicate measurements are often necessary for confidence in biologics quantification. Homebrew streamlines the process of evaluating replicates by providing built-in statistical tools. Give your replicate samples the same name and you can make stats a snap. An easy-to-access interface means you don't need hours of training to design your applications in Lunatic & Stunner Client and lets you control the data for your molecule of interest, be it protein, DNA, RNA, or something else (Figure 2).

Homebrew's Settings tab lets you pick your sample type to keep your applications organized. Data can be recalculated between applications for the same sample type, so you can measure samples with a default Lunatic protein application, then turn around and re-analyze the same data in a Homebrew protein application, saving you time and sample volume. Sample type also determines if you need to input a protein extinction coefficient or if the software will use a nucleic acid concentration factor.

While Stunner and Lunatic determines sample absorbance across the entire UV/Vis spectrum, sometimes only a single wavelength or range is relevant to your Homebrew assay. The wavelength of interest gets displayed on the results table in Lunatic & Stunner Analysis while the visible wavelength scale determines the x-axis of the displayed absorbance spectrum.

Homebrew applications allow you to choose background correction options to give you control over your results. Turbidity correction uses Rayleigh scattering to apply wavelength-specific correction factors to the absorbance spectrum. Intelligent single point correction tells the software to find a wavelength with no absorbance to use for background correction, ensuring absorbance values are never negative. Homebrew also supports user-defined single point background correction so it can be compared to other UV/Vis spectrophotometers. Homebrew can apply correction equal to the average absorbance in a wavelength range, typically used for colored samples with a well-defined transparent wavelength range. Lastly, you can choose no background correction at all.

Methods

Bovine IgG (bIgG) was prepared at 1 and 10 mg/mL in phosphate-buffered saline (PBS) and named

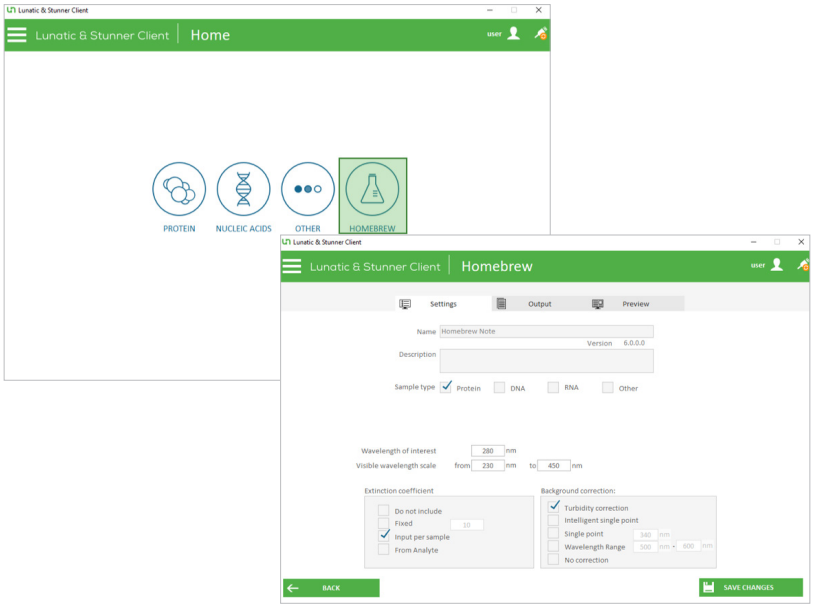

Figure 2: Homebrew on Lunatic and Stunner features multiple sample type categories, custom wavelengths of interest, user-defined spectral range, several extinction coefficient options, and a slew of background correction options.

Sample A and Sample B, respectively. 2 µL of each sample and PBS as a blank were loaded in triplicate into a High Lunatic plate. The absorbance spectra, concentrations, averages, standard deviations (SDs), and coefficients of variation (CVs) were determined using a Homebrew protein application built in Lunatic & Stunner Client v6.0 with turbidity background correction and an inputted E1% of 13.7.

Results

The Output tab of Homebrew allows the creation of user-defined outputs and offers easy access to statistical, arithmetic, or algebraic functions for built-in or user-defined variables (Figure 3). By default, protein Homebrew applications contain a single output called Concentration that is determined from the background-corrected absorbance at 280 nm and the E1% as follows:

$$Concentration = \frac{A280}{E1\%} * 10$$

E1% can be calculated from the molar extinction coefficient (ε) and the molecular weight (MW) of a protein as follows:

$$E1\% = \frac{\varepsilon \times 10}{MW}$$

Additional outputs can be created using the variable buttons in the middle of the screen and the function buttons on the right side of the screen. Outputs are listed in the Output box on the left side of the screen.

Outputs for the average, SD, and CV of concentration were created in this application using the built-in statistical functions. These values were calculated for wells with the same sample name and displayed along with the absorbance spectrum in Lunatic & Stunner Analysis (Figure 4).

Homebrew on Stunner

DLS does viscosity

The high viscosities inherent to high-concentration biologics formulations present significant challenges for manufacturing and efficacy. Traditional viscometers consume large amounts of sample, which usually necessitates later characterization. The Homebrew toolkit on Stunner allows for a DLS-based method of determining viscosity. This high-throughput method uses low sample volumes, allowing earlier assessment of formulation effects and establishing when higher concentrations of proteins start to impact viscosity. This means you can get around the limitations of traditional viscometers and get a read on viscosity at an earlier

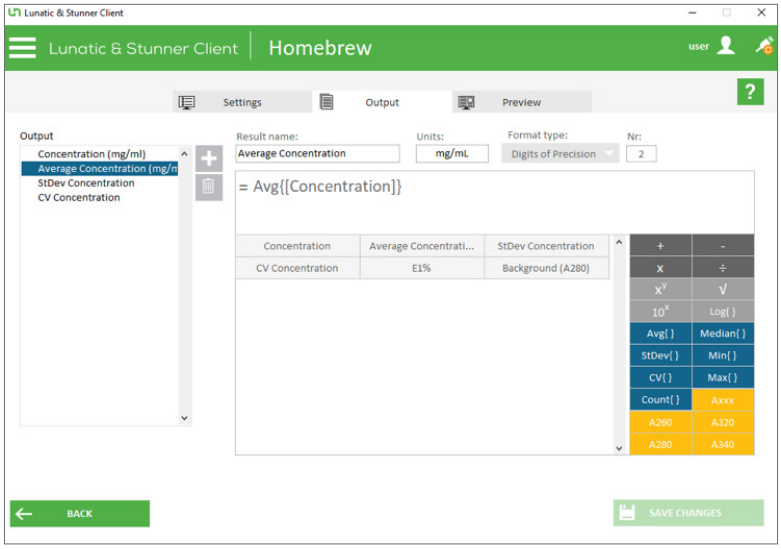

Figure 3: Homebrew Output tab offers statistical calculations (blue buttons) right at your fingertips. It also allows arithmetic (dark grey buttons) and algebraic (light grey buttons) functions. Incorporating absorbances at specific wavelengths (yellow buttons) into your calculations is a snap.

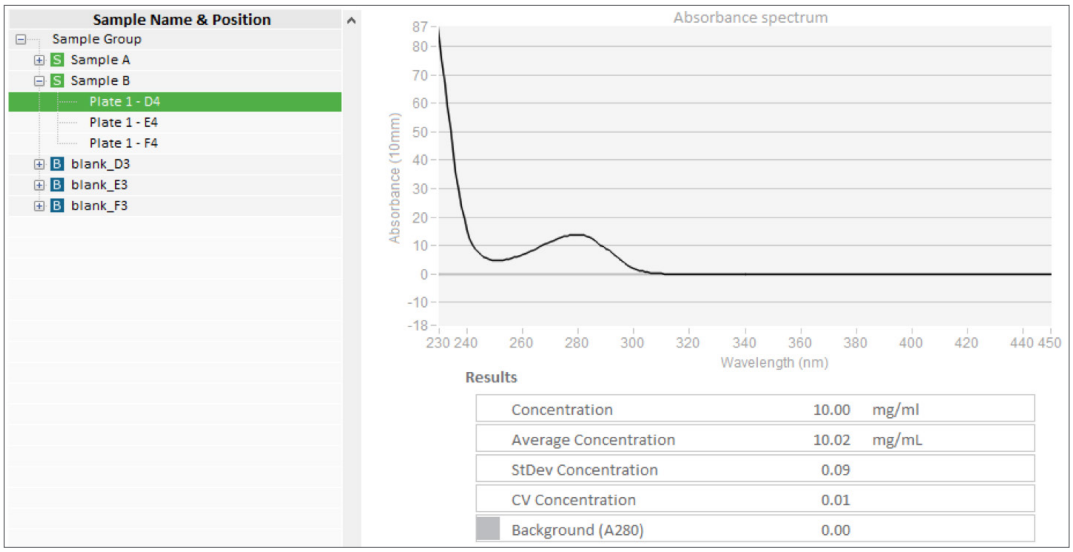

Figure 4: Concentration, average, SD, and CV of replicates of 10 mg/mL bIgG in PBS are displayed in the Results table of this Homebrew application, along with the absorbance spectrum. Statistics were calculated for all samples sharing the same name.

stage in your process for more formulations. When combined with Stunner's B_{22} & k_D application, viscosity with Homebrew provides a fast and easy method to determine aggregation propensity of your biologic with a single instrument.[2]

Homebrew in Stunner has all the same tools as Homebrew in Lunatic, with one big extra. It allows you to include DLS sizing results and specify the sizes of interest in the Settings tab (Figure 5A). You can quickly use these variables in the Outputs tab with a touch of a button with any of the Homebrew functions (Figure 5B).

Methods

100 nm NIST standard beads were added to samples of 0%, 6%, 13%, 20%, 25%, and 33% m/v glycerol in PBS or samples of 0, 0.5, 3.2, 25, 50, and 100 mg/mL monoclonal antibody (IgG) in 20mM His-HCl, 150 mM Arg-HCl, pH 6. 2 µL of the bead-containing samples and bead-free samples were loaded in triplicate in a Stunner plate. Diffusion coefficients (D) were measured for each sample using 4 DLS acquisitions of 5 seconds each. A Homebrew application calculated sample viscosities with these results along with the Boltzmann constant (k_B), measured temperature

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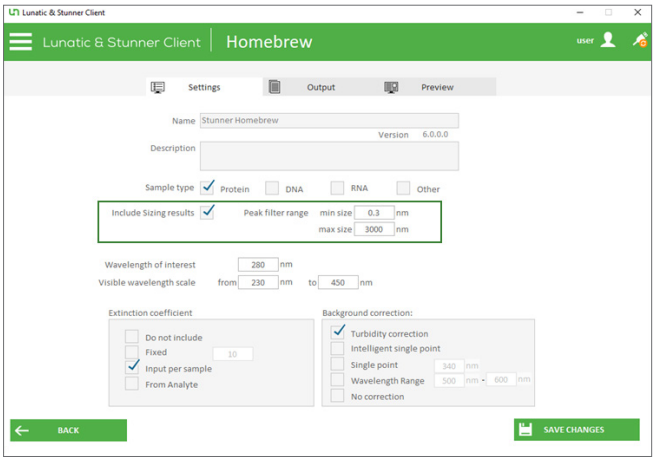

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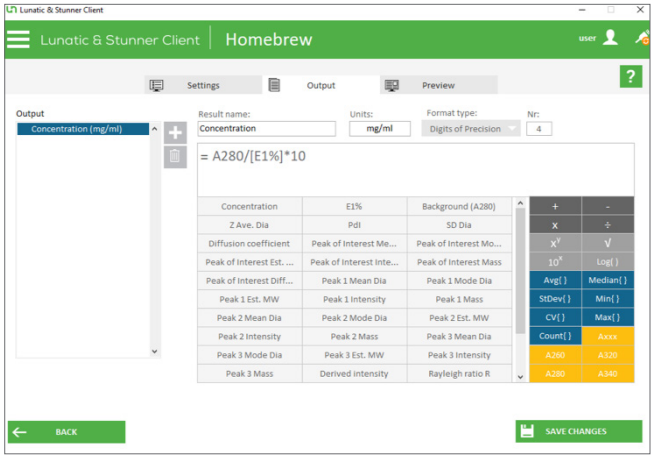

Figure 5: Homebrew in Stunner lets you include sizing and polydispersity results into your Results table (**A**) or incorporate DLS results as variables into your outputs (**B**).

(T), and known average bead radius (R_H) using a re-arrangement of the Einstein-Stokes equation as follows:

$$\text{Viscosity} = \frac{k_B T}{6\pi D R_H}$$

The average and standard deviation of the viscosity, and the intensity distribution of each sample was also determined by the Stunner Homebrew application. The viscosities of IgG samples were also quantified with a RheoSense µVISC™ viscometer.

Results

When the viscosity of a solution increases, the diffusion coefficients and apparent size of particles in that buffer also increase. By adding NIST standard beads to a solution Stunner can use a Homebrew application to determine the viscosity of the solution with the measured diffusion coefficient and the known size of the beads. Samples of PBS with increasing concentrations of glycerol had increasing viscosities (Figure 6). Viscosities determined by Stunner with the Homebrew application had close agreement with published viscosity values.[3]

Monomeric IgG particles have an approximate average size between 10–20 nm and are easily distinguishable from 100 nm NIST standard beads by DLS on Stunner (Figure 7A). Homebrew allows the user to specify peaks of interest and was used to isolate the diffusion coefficient value for the bead peak (green box) from the antibody peak (blue box) for viscosity calculations. The diffusion coefficient of the beads was then used to determine the viscosity of the antibody solution (Figure 7B). Viscosity values from Stunner agreed closely with values measured by a viscometer.

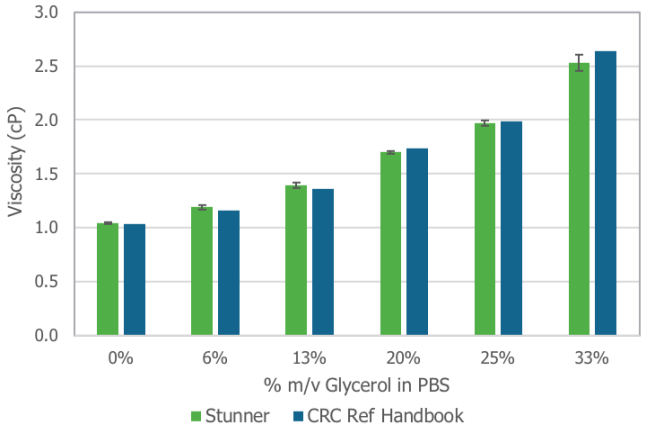

Figure 6: Viscosity of PBS with different concentrations of glycerol determined with 100 nm NIST standard beads by DLS in a Stunner Homebrew application. Stunner values (green) closely agree with published values (blue).

Conclusions

With the capability to quantify highly concentrated samples in a dilution-free and reproducible manner, Lunatic is a game-changer for protein quantification. Stunner combines the same UV/Vis spectral analysis with DLS for quantifying concentration and assessing protein quality on one platform. Both platforms are high-throughput with low sample volumes of only 2 µL, saving you time and sample. Lunatic and Stunner provide you

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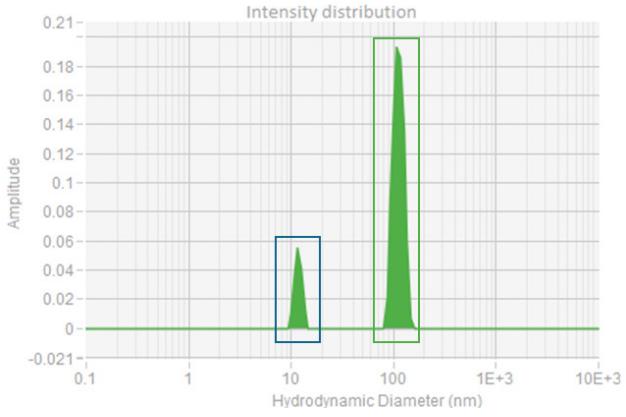

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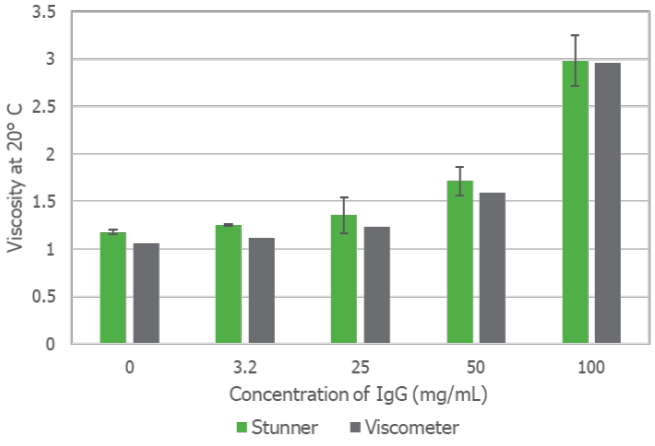

Figure 7: Example intensity distribution of 100 nm NIST standard beads in 0.5 mg/mL IgG solution showing peaks from the antibody (blue box) and beads (green box) (**A**). Viscosity, determined by Stunner and a viscometer, of several concentrations of IgG in 20 mM His-HCl, 150 mM Arg-HCl, pH 6.0 (**B**).

with the entire UV/Vis absorbance spectrum and versatile options for background correction, giving you more control over how your data is handled. The Homebrew toolkit on both Lunatic and Stunner augments their already impressive flexibility with an easy-to-use interface that allows you to design your own applications. Homebrew makes statistics a snap on either Lunatic or Stunner, allows you to measure viscosity by DLS on Stunner, or lets you build your own custom equations. With Homebrew, the only limit is your imagination.

References

1 Weak interactions govern the viscosity of concentrated antibody solutions: High-throughput analysis using the diffusion interaction parameter, B Connolly, et al., Biophysical Journal, 2012; 103(1):69–78.

2 Spot aggregation early with B_{22} and k_D on Stunner, Application Note, Unchained Labs, 2019.

3 CRC handbook of chemistry and physics: a ready-reference book of chemical and physical data, 95th Edition, W Haynes, Boca Raton: CRC Press, 2014.

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