Use of Gen5™ Discontinuous Kinetics for Long Term Kinetics

Monitoring Algal Cell Growth for Biofuel Applications

Long term growth measurements usually require that the cultures be maintained under optimal or desired growth conditions with an aliquot tested periodically as the microplate reader may not be ideal for optimal growth. For example, algal cultures often require polychromatic light for photosynthetic growth, while the read chamber of a microplate reader is normally dark and light tight by design. Also, cell cultures demand humidity levels approaching 100% to prevent evaporative loss. In addition, long-term growth monitoring often only requires one or two measurements per day for a period of weeks. Dedicating a microplate reader for such a period of time at the exclusion of all other experiments is not practical. Budgetary constraints being what they are dictate that providing a second dedicated reader to be cost prohibitive. The traditional solution has been to read the samples as a traditional endpoint read and collate the data manually at the completion of the experiment. The use of the **Discontinuous Kinetics Option** in Gen5 Data Analysis and Reader Control Software offers a solution to this problem.

Discontinuous kinetics provides a means for a long-term kinetic reading with multiple measurements on a plate or a series of plates over any period of time without tying up the reader. This option allows removal of the plate for processing after each read interval, so other experiments can be conducted with the reader in-between read intervals. When enabled, the Discontinuous Kinetics option uses the internal clock of the PC to provide a time and date stamp to each read event, allowing Gen5 to keep track of the total run time of the entire assay.

Selecting Discontinuous Kinetics

In order to use the discontinuous kinetics option a New Experiment file needs to be created that either enables the feature directly or uses a preexisting Gen5 Protocol file with the feature enabled. It is critical that this option is enabled prior to running the assay. Discontinuous kinetics is enabled from the **Advanced Options** button or Options link located in the **Protocol Procedure** window.

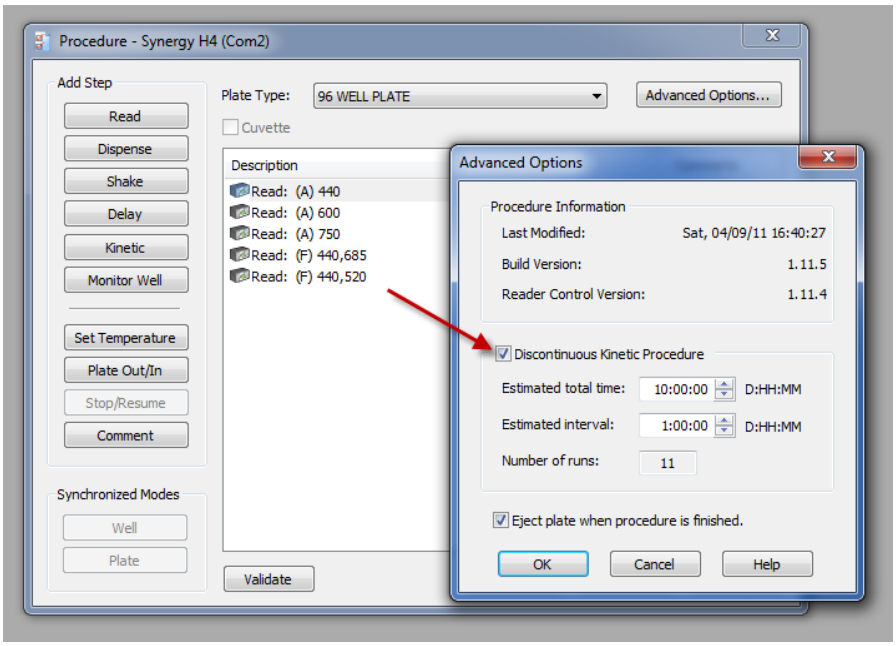

Figure 1. Advanced Options Window

In the Advanced Options window is a check box to enable discontinuous kinetics (Figure 1 red arrow). Once Discontinuous kinetics has been enabled, Gen5™ requires that an "estimated" number total run time Days:Hours:Minutes as well as an expected read interval be entered. Note that these are just an estimate and will not interfere with actual experiment activity. Gen5 uses your estimated time lines to set up the data views options and formulas with placeholders until the actual data is captured. When in doubt about the required time period and intervals, it is best to **overestimate** them, such that the number of read value is maximized. Note that the “Eject plate when procedure is finished” option is enabled. This option (when supported by the reader) gives you more control over plate processing. The default setting mirrors most BioTek readers: it ejects the plate when the Read/Procedure is completed. If a situation demands that the plate remain inside the reader upon the completion of the read, one can de-select the option to keep the plate inside the reader at the end of a Procedure.

Example

Chlorella vulgaris algae growth can be used as an example to demonstrate the Discontinuous Kinetics Procedure. As demonstrated in Figure 2, the growth of this single cell algal strain was monitored over a period of 17 days using light scattering absorbance as an indicator of cell growth. Over time the measured absorbance at 600 nm increases indicating an increase in cell number. Note that while a single measurement was taken daily, the data points are not at evenly spaced intervals, indicating that plate measurements were not necessarily at the same time each day or that a measurement point was missed.

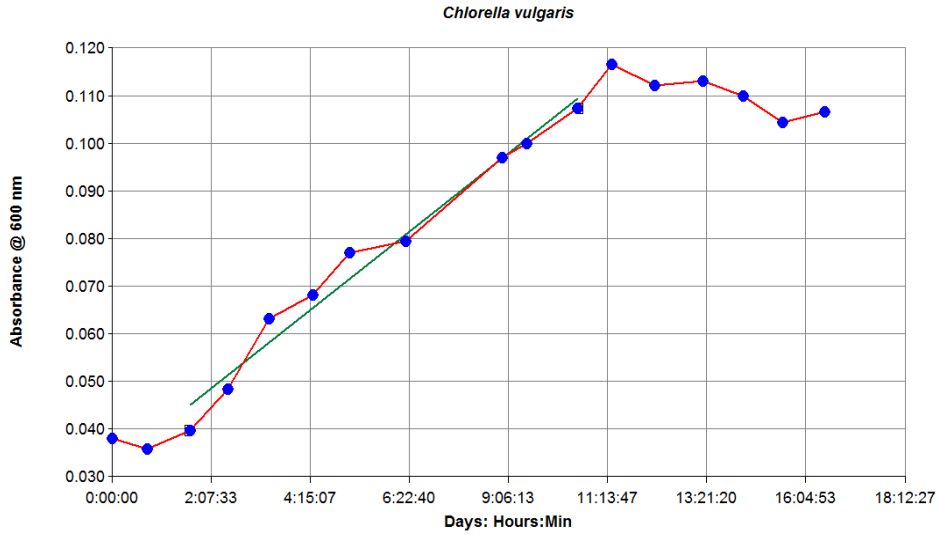

Figure 2. Gen5 kinetic plot of *Chlorella vulgaris* algae growth. Gen5 discontinuous kinetics procedure was used to maintain a data timeline. A mean slope value (Green line) was determined from data points 3-11.

Discussion

The purpose of discontinuous kinetics is to link a series of endpoint measurements together in regards to time in a manner similar to traditional kinetic measurements and provide the same data reduction tools. Discontinuous kinetics has several features that are distinctly different from traditional kinetics. As previously stated discontinuous kinetics allows the user to make kinetic measurements over a long period of time without tying up the reader during the intervals between reads. In addition it also allows flexibility in the read interval. Because the plate read is initiated manually the time between reads is not rigidly fixed. Data is plotted based on the total run time at the time of the plate read. All kinetic calculations are plotted as a function of the recorded time. This allows for calculations such as mean slope V or maximum slope V to be automatically determined by Gen5™ regardless of the actual measurement interval. The discontinuous kinetics procedure can also be used to monitor several plates over time. This is particularly advantageous when a BioTek reader is used in conjunction with a BioStack™ Microplate Stacker. Several plates can be read in sequence for any number of cycles without any manual intervention, with kinetic curves being generated for each well of each plate automatically by the Gen5 Data Analysis Software.