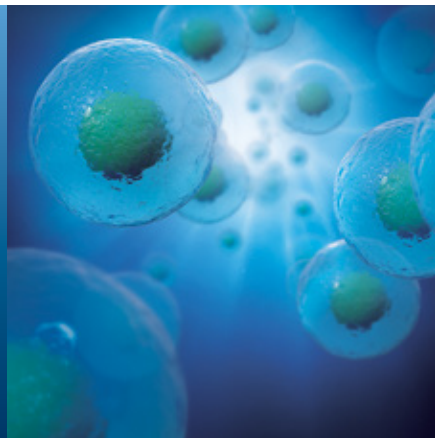
HAMILTON

Incyte Correlation

Demo Protocol



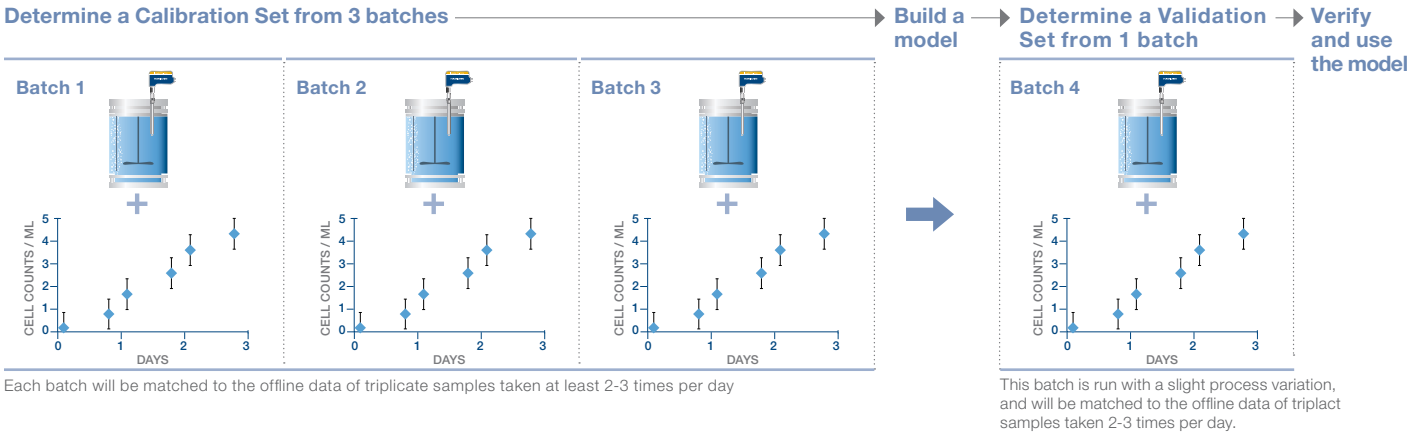
Introduction

The goal of this protocol is to support users of Incyte in obtaining relevant data for the correlation of viable cell density with offline measurement. Two methods may be used to correlate online cell density data with offline cell measurement: 1) a simple linear correlation, and 2) advanced Multivariate Analysis. The linear correlation is an uncomplicated method that is ideal for the exponential phase of growth. However, it overestimates the cell density during the lag and the death phases. Multivariate Analysis, using Partial Least Squares modeling, provides a better correlation across the complete process. The experimental setup is summarized in Figure 1.

Method 1: Linear Correlation

1. Prepare the culture.
2. Start recoding and perform a product calibration (Cell Density Monitoring System Operational Manual, Chapters 7.1 to 7.4).
3. Optional: Activate the Incyte Scan and perform the Incyte Scan product calibration (Chapter 7.12.2). This step is only required if you want to perform a Multivariate Analysis at a later stage.
4. Inoculate and set the inoculation time (Chapter 7.4).
5. Take regular samples 2-3 times per day. Take 3 separate samples each time a regular sample is taken. When taking the samples, mark the sampling time in the Arc View Controller by adding the comment ‘samplen’ with ‘n’ being the sample number (Chapter 7.5). We recommend using an impedance based cell counter such as CASY or Coulter Counter for offline measurement. In addition to measuring the cell counts, an analysis of the average cell diameter is recommended.
6. If only one sample was taken for each regular sample at Step 5, repeat the offline measurement 3 times. Enter the results from the 3 samples in the provided Excel spreadsheet and determine the average. (Figure 2).
7. Export the culture file (Chapter 7.10).
8. Repeat the culture two times (Steps 1-7).

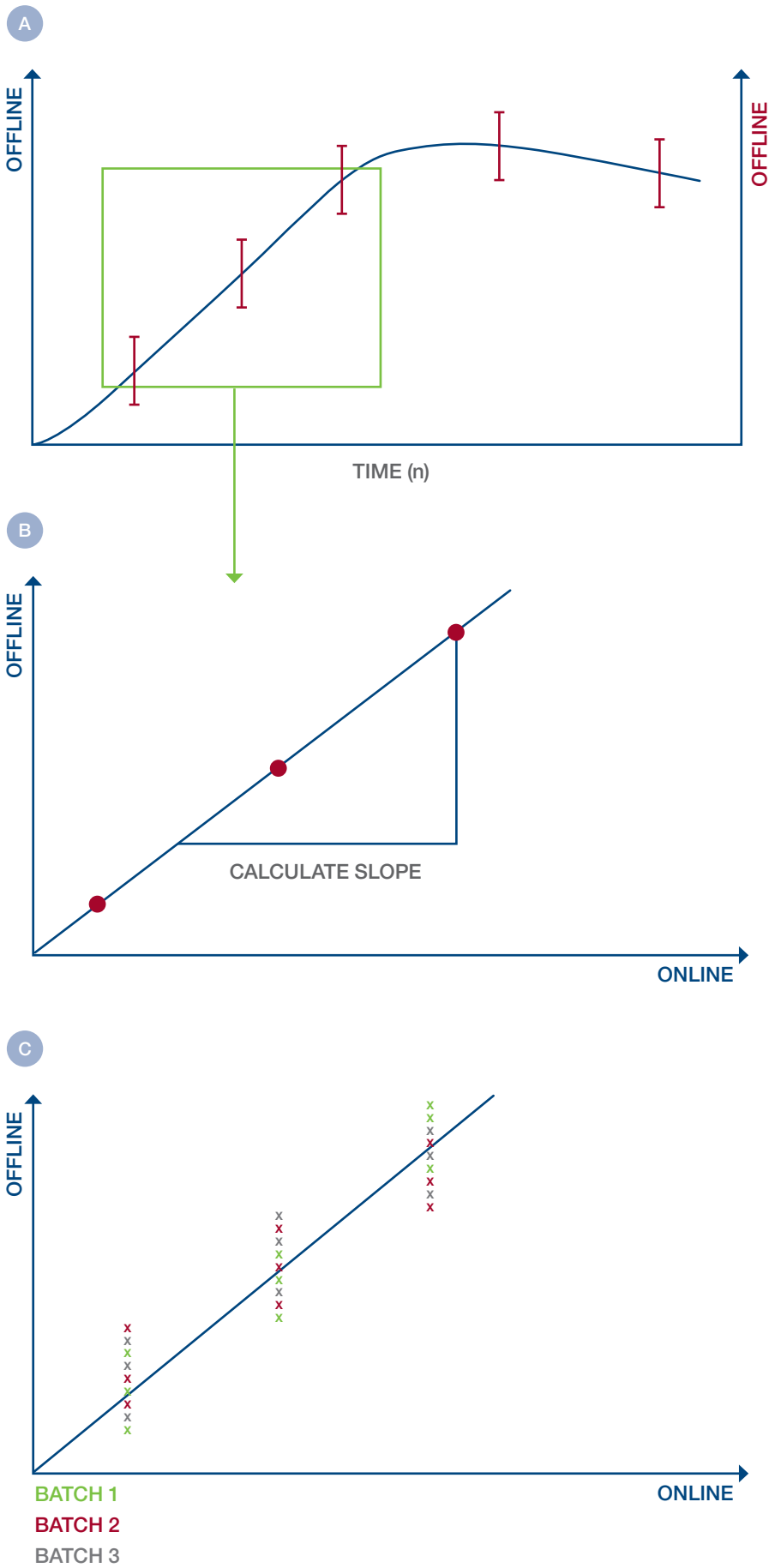
Figure 1: *Experimental setup*



9. Plot the online and offline cell density measurements against time for comparison (Figure 2A).
10. Identify the measurements within the exponential phase (Figure 2A, green square). Plot the offline measurements of the exponential phase against the online measurements and determine the correlation factor (Figure 2B), using the data of all three batches (Picture 2C). Calculate the slope (= cell factor). Note: Cell density is usually overestimated in the lag and death phases using this method.
11. To graph the data on the Excel file, perform the following steps:

- **Load your data into the Excel spreadsheet as described in the Operating Instructions (Chapter 7.11).**
- **Create an X-Y Diagram with interpolated lines.**
- **Use the culture time as x-axis and the permittivity measurement as y-axis.**
- **Plot the offline data on the secondary y-axis.**
- **Define the exponential growth phase.**
- **Use the Excel function =SLOPE (y value, x value) to calculate the slope of the corresponding data from the growth phase.**

Figure 2: Linear data correlation, for one and three batches



Method 2: Preparation for Multivariate Analysis

This method requires repeating the culture process three times to acquire enough data to determine a calibration set. A fourth batch with a slight variation (e.g. run at a lower pH) is needed to verify the calibration set (Figure 1). We recommend sampling two to three times per day to obtain a good correlation for all phases of the process. This analysis is not yet covered in the Arc View Controller Software. Therefore, both the data analysis as well as the model building must be done on suitable PC Multivariate Analysis platforms, such as Eigenvector or Matlab. Creating a model based on three fermentations generally creates a good correlation on the overall bioprocess, but may be refined over time based on user experience.

1. Prepare the culture.
2. Begin recording and perform a product calibration (Cell Density Monitoring System Operational Manual, Chapters 7.1-7.4).
3. Activate the Incyte Scan and perform the Incyte Scan product calibration (Chapter 7.12.2).
4. Inoculate and set the inoculation time (Chapter 7.4).
5. Take regular samples 2-3 times per day. Take 3 separate samples each time a regular sample is taken. When taking the samples, mark the sampling time in the Arc View Controller by adding the comment 'samplen' with 'n' being the sample number (Chapter 7.5). We recommend using an impedance based cell counter such as CASY or Coulter Counter for offline measurement. In addition to measuring the cell counts, an analysis of the average cell diameter is recommended.
6. If only one sample was taken for each regular sample at Step 5, repeat the offline measurement 3 times. Enter the results from the 3 samples in the provided Excel spreadsheet and determine the average. (Figure 2).
7. Export the culture file (Chapter 7.10) and import it into the provided Excel spreadsheet (Figure 3).
8. Repeat the culture two times (steps 1-8).
9. Prepare a fourth culture introducing slight variation, e.g. vary the process pH by +/- 0.3 pH values. Additional batches from the same culture may be used to verify and confirm the correlation model.
10. Export the culture files (Chapter 7.10) to your PC. If desired, email both the culture files and the offline measurements to Hamilton for verification of the model. We can also perform the Multivariate Analysis if requested.
11. Note: Alternatively you may use standard statistical software with PLS functionality (e.g. Matlab, Eigenvector) to perform the data correlation.

Figure 3: Offline Measurement

Time Point	Measurement 1	Measurement 2	Measurement 3	Average	Standard Deviation
Sample1	3.48	3.48	3.48	3.48	0
Sample2	5.60	5.60	5.60	5.60	8.88178E-16
Sample3	8.39	8.39	8.39	8.39	0
Sample4	14.11	14.11	14.11	14.11	0
Sample5	20.77	20.77	20.77	20.77	0
Sample6	33.65	33.65	33.65	33.65	0
Sample7	48.38	48.38	48.38	48.38	0
Sample8	75.11	75.11	75.11	75.11	0
Sample9	95.98	95.98	95.98	95.98	0
Sample10	126.09	126.09	126.09	126.09	1.42109E-14
Sample11	113.70	113.70	113.70	113.70	0
Sample12	80.53	80.53	80.53	80.53	0

Figure 4: *Data from the Arc View Controller*

- Creation Date: Mon May 19 15:38:24 2014
- Batch Number: 03-48
- Acquisition time: 720 s
- Original file name: RT5_WP1_DoE_Run3_Ch3.xls
- Cell Line: CHO
- Operator: MBR
- Comment:
- Probe Status: OK
- Probe calibration date: 2014-04-18
- Amplifier serial number: PRE-7DC-143
- Sensor serial number: D12-7DA-328
- Zero: 0.15
- Biomass unit/factor: 1.00
- Measure mode: Animal cell culture . fmes:1000.00kHz. fhigh:10000.00kHz . integration:HIGH

Date	Record Time(h)	Culture Time(h)	Comment	Conductivity (mS/cm)	Permittivity (pF/cm)	Biomass	fc(kHz)	DeltaEsp (pF/cm)	Alpha	C(300kHz)	C(373kHz)	C(465kHz)	C(8031kHz)	C(10000kHz)	Offline Measurement
5/19/14 15:38	0.00		Start recording	10.15	0.30	0.14	500.00	0.61	0.10	-1.04	-0.48	-0.16	0.23	0.24	
5/19/14 15:38	0.00	0.00	Inoculation	10.15	0.30	0.14	500.00	0.61	0.10	-1.05	-0.48	-0.16	0.23	0.24	
5/19/14 15:50	0.20	0.20		10.17	0.30	0.30	500.00	1.62	0.10	0.50	0.30	0.14	-0.77	-0.74	
5/19/14 16:02	0.40	0.40	Sample1	10.18	0.30	0.30	500.00	1.62	0.10	0.47	0.28	0.12	-0.76	-0.75	3.48
5/19/14 16:14	0.60	0.60	Sample1	10.19	0.30	0.30	500.00	1.63	0.10	0.49	0.28	0.13	-0.77	-0.76	3.48
5/19/14 16:26	0.80	0.80	Sample1	10.20	0.32	0.32	500.00	1.55	0.10	0.53	0.32	0.15	-0.77	-0.74	3.48
5/19/14 16:38	1.00	1.00	Sample1	10.19	0.31	0.31	500.00	1.68	0.10	0.52	0.32	0.16	-0.76	-0.73	3.48
5/19/14 16:50	1.20	1.20	Sample1	10.20	0.30	0.30	500.00	1.65	0.10	0.54	0.34	0.16	-0.76	-0.73	3.48
5/19/14 17:02	1.40	1.40	Sample1	10.21	0.30	0.30	500.00	1.67	0.10	0.55	0.33	0.18	-0.75	-0.73	3.48
5/19/14 17:14	1.60	1.60	Sample1	10.21	0.31	0.31	500.00	1.65	0.10	0.53	0.32	0.18	-0.72	-0.72	3.48
5/19/14 17:26	1.80	1.80		10.21	0.32	0.32	500.00	1.58	0.10	0.55	0.34	0.17	-0.74	-0.69	
5/19/14 17:38	2.00	2.00		10.22	0.32	0.32	500.00	1.66	0.10	0.53	0.34	0.20	-0.72	-0.69	
5/19/14 17:50	2.20	2.20		10.21	0.33	0.33	500.00	1.77	0.10	0.53	0.33	0.20	-0.71	-0.68	

Discover More

- Please visit the product website for more information: http://www.hamiltoncompany.com/products/process-analytics/sensors/cell-density
- For assistance on the Multivariate Analysis, please contact: techsupport.pa.ch@hamilton.ch.

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