	Time	rpm	Temp°C	DO1	DO2	pH1	pH2	load	YSI glu	OD	Ref glu	OD Hg
No spent LB	21:11	691	30.2	68.3	68.9	7.01	6.98	10.87	16.3	14.5		
①	22:00	692	30.3	42.3	39.7	7.00	6.97	10.86	10.8	22.0	~~2.41~~	
② ③	22:17	691	30.3	42.3	39.5	7.00	6.97	10.81	~~2.42~~ 9.06	24.0	2.42	
④	22:35	691	30.1	46.2	43.8	7.01	6.97	10.99	16.76	25.6	2.46	
	22:55	691	30.2	38.4	35.4	7.01	6.97	10.88	16.6	25.4	2.42	
	23:11	691	30.1	44.7	42.3	7.01	6.98	10.88	16.9	26.6	2.48	.865
	23:19	692	30.1	47.9	45.7	7.01	6.98	10.83	15.6	30.8	2.42	.865
	23:39	693	30.2	50.9	49.0	7.01	6.97	10.80	14.0	25.9	2.43	
⑤	23:54	694	30.0	56.6	55.1	7.01	6.98	10.75	11.9	25.5	2.43	
LB	00:14	694	30.2	59.4	58.1	7.01	6.97	10.73	11.8	34.0	2.51	37.0
	00:32	693	30.1	61.1	59.9	7.01	6.98	10.76	10.4	36.7x3	2.50	
	01:01	693	30.1	64.9	64.3	7.01	6.97	10.57	8.15	16.6x2	~~2.23~~	
⑥ ⑦	×	×	×	×	×	×	×	×	8.19	17.2x2	0.00	
	01:28	693	30.3	67.6	67.1	7.01	6.98	10.51	×	32.8	× 865	
	01:46	693	30.1	69.0	68.6	7.01	6.98	10.49	5.41	27.9	2.24	

MEETING WITH BIOCONTROL AND STEVE DURFEE
June 12, 1997

General Business Discussion:

1. This is an exclusive marketing and development agreement between Biocontrol Technology and Steve Durfee to use Biocontrol's infrared spectrophotometer to measure chemical components in fermentors and bioreactors. Both parties have verbally agreed that after checking with their legal representatives, mutually acceptable changes can be made to the agreement.

2. This is a joint venture between Biocontrol and Steve Durfee and an investor yet to be determined. If Steve Durfee so chooses, he may reopen the current agreement for discussion of consideration of another business arrangement.

Items to occur before the agreement becomes a joint venture:

1. Steve Durfee will complete a market analysis.

2. Steve Durfee will find a source of funding (venture capitalist or other).

3. Biocontrol and Steve Durfee will work together to demonstrate feasibility.

General Development Discussion:

1. Biocontrol will assemble a probe for Steve Durfee to perform initial exploratory testing.

   Dave Purdy: Steve's business plan should include this initial exploratory phase using a probe and instrument provided by Biocontrol.

2. It is agreed that Biocontrol and Steve Durfee at this time are willing to provide minimal funds to finance exploratory testing.

3. Biocontrol will loan a modified D-1000 instrument, probe, and laptop computer to Steve Durfee free of charge for the feasibility demonstration.

   Steve Durfee has agreed that he will not demonstrate the instrument provided by Biocontrol to anyone. Steve Durfee will not leave the instrument anywhere unattended and it will be under his constant supervision.

   Dave Purdy will check with the Biocontrol directors about allowing Steve to have a D-1000 instrument.

Biocontrol may send someone with the instrument to Steve Durfee's laboratory of choice, or Steve Durfee may come to Indiana to do the testing at Biocontrol's laboratory.

4. If testing is successful, it will aid in raising funds for development.

Market and Product Development Agreements:

1. Probe: Biocontrol will build a new probe at least two feet long on one of the E-prototype units and provide a manual reference. Biocontrol will update the D-1000 instrument with the latest improvements affecting its ability to measure glucose.

2. Software: Tom Kerr of Biocontrol will assemble modified data acquisition software. He will include instructions for using the modified D-1000 instrument and laptop computer.

3. Hardware: Biocontrol will loan a modified D-1000 instrument with probe and laptop computer to Steve Durfee for exploratory testing.

4. Testing: Steve Durfee will provide information to Todd Barker for design of experiment for exploratory testing and determination of a calibration vector. This information will be used to collect data in order to obtain a calibration.

5. Algorithm: Biocontrol will provide temporary use of Matlab and other PLS software to Steve Durfee for developing the calibration vector and prediction algorithm during exploratory testing. Todd Barker will verify calibration and help build prediction algorithm.

6. Verification: Steve Durfee will repeat testing to verify calibration.

7. Steve Durfee will send a sample of typical fermentation broth to Pete Raber of Biocontrol for determination of optical properties.

   Steve commented that the broth is not particularly hazardous (do not eat or breathe); the organisms are dead.

8. Cost: Biocontrol will determine a cost estimate of the exploratory phase.

9. Schedule: Dave McMurry will provide a schedule to provide initial test instrument. Steve Durfee will use this to determine an overall exploratory testing schedule.

/lb

BIOCONTROL
TECHNOLOGY INC.

Biocontrol Technology, Inc.
300 Indian Springs Road
Indiana, PA 15701

FAX COVER SHEET

Date: 12/5/97

Number of pages including cover sheet: 1

To: Steve Durfee

From: Jeremy A. Grata

Phone: 303 772 1041

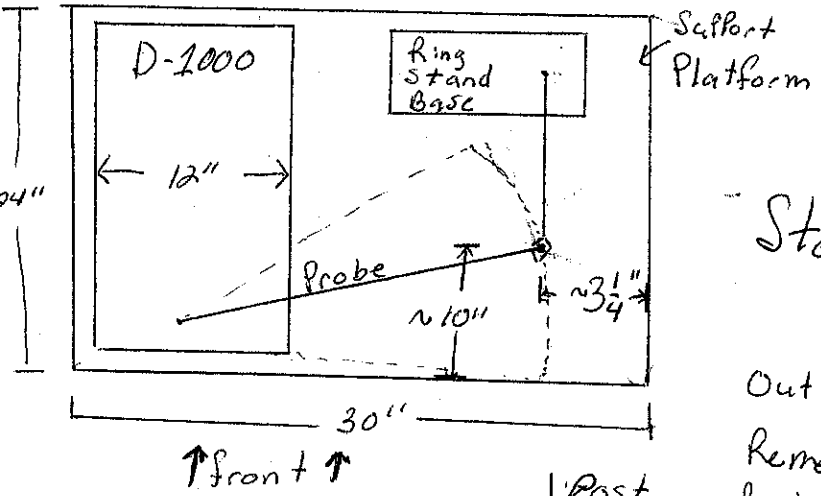
Phone: 412-349-1811x142

Fax Phone: 303 772 1042

Fax Phone: 412-349-8610

CC:

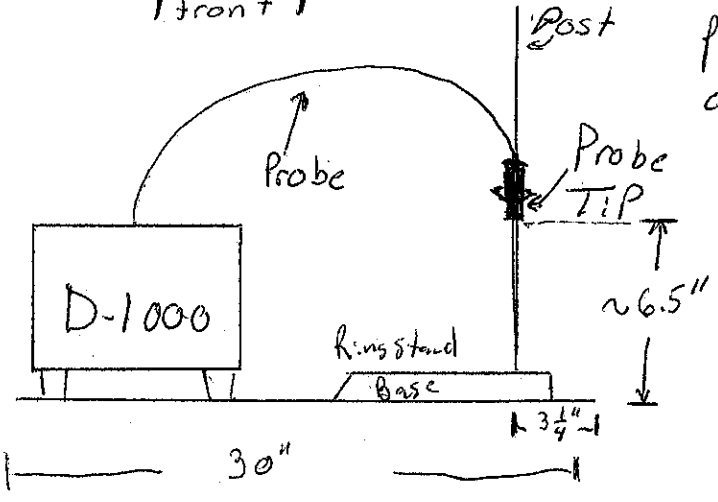
Remarks: ☐ Urgent ☑ For your review ☐ Reply ASAP ☐ Please comment



Top View

Steve,

The area that the probe would map out on the support platform may be Removed. The only Requirement is that the probe Tip be secured to the same platform as the D-1000.



Front View

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0908411.001
GLUCOSE & SPECTROSCOP--

BDL ISS 97-48
EXP ISS 12-02-97
EPA ISS 97-48
EPB ISS 97-48
INS ISS 97-48
PAT ISS 12-02-97
PCT ISS 11-27-97
PCT ISS 12-04-97

Citations from INSPEC: INS ISS 97-48

1. NDN 083-0576-9416-0: Raman measurement of glucose in bioreactor materials

1. Raman measurement of glucose in bioreactor materials - INS 97-48 5769417 A9802-87808-005 (PHA) NDN- 083-0576-9416-0

Yijin Xu; Ford, J. F.; Mann, C. K.; Vickers, T. J.; Brackett, J. M.; Cousineau, K. L.; Robey, W. G.

JOURNAL NAME- Proceedings of the SPIE - The International Society for Optical Engineering ABBREVIATED JOURNAL TITLE- Proc. SPIE - Int. Soc. Opt. Eng. (USA) VOL. 2976 1997 PP. 10-19 15 reference(s) DOCUMENT TYPE- Journal paper ISSN- 0277-786X CODEN- PSISDG CORPORATE AUTHOR- Dept. of Chem., Florida State Univ., Tallahassee, FL, USA COPYRIGHT OF BIBLIOGRAPHIC- Copyright 1997, IEE COPYRIGHT CLEARANCE CENTER CODE- 0277-786X/97/$10.00 PUBLISHER- SPIE-Int. Soc. Opt. Eng PUBLICATION COUNTRY- USA CONFERENCE TITLE- Biomedical Sensing, Imaging, and Tracking Technologies II S I C I- 0277-786X(1997)2976L.10:RMGB;1-A LANGUAGE- English (DEF)

The feasibility of using Raman spectroscopy to monitor the concentration of chemical species in a bioreactor has been examined. Successful operation of a bioreactor requires that nutrients and metabolic waste products be maintained within narrow ranges, and it is, therefore, important to provide accurate, reliable and timely measurement of the composition in the reactor. Raman spectroscopy offers the possibility of real time simultaneous monitoring of molecular components present in the millimolar and higher concentration range. Work reported here has focused on four analytes: glucose, glutamine, lactic acid and ammonia. Measurements have been made with a spectrograph providing a spectral window for simultaneous measurement of about 1800 cm/sup -1/ on a multichannel CCD detector. Most measurements were made with an argon ion laser emitting at 514.5 nm. Some measurements are reported with a solid state diode laser operating at 785 nm. Locally constructed inexpensive silica fiber-optic probes delivered the laser light and collected the scattered radiation. Spectra of the four analytes in buffer and reactor media have been obtained. Analytical curves have been constructed and limits of detection measured. Limits of detection in buffer media are about 1 mM. Results are reported for off-line measurements on material drawn from a bioreactor.

HIT TERMS: GLUCOSE?; SPECTROSCOP?.

Strategy Number: 01 Strategy Date: 01/17/96 TS: GLB Hit Limit: 50 Copies: 1

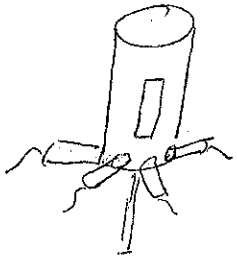
1 01 98 GLUCOSE?\TD
2 99 98 SPECTROSCOP?\AL

Hits in this report for strategy 01:

BDL ISS 97-48	0 hits
EXP ISS 12-02-97	0 hits
EPA ISS 97-48	0 hits
EPB ISS 97-48	0 hits
INS ISS 97-48	1 hits
PAT ISS 12-02-97	0 hits
PCT ISS 11-27-97	0 hits

D1000 - Installation & Assembly Good (~5:00pm Wed MT)

Fermentor:



Continuous monitoring of pH
Range 7.00 ~ ±.02 fixed

Dissolved Oxygen 20 ~ 80 ±.1
Used to determine metabolic mode of bacteria (Less DO, more growth)

Temperature ~30°C Range ~± 10°C ±.01
Used for different growth specifications fixed

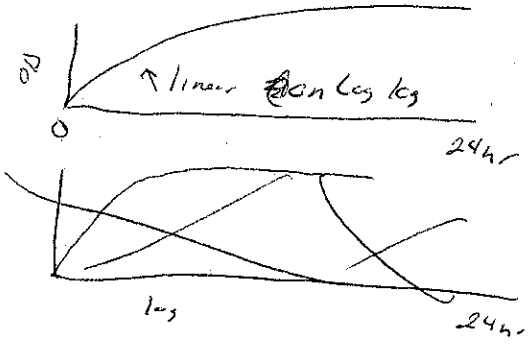
Volume of Solution depends on fermentor size & desired volume of Bacteria
2L - 150L

pH & DO Have 2 probes each for reliability 25 mm standard insert

Technicians performed tests

Optical Density: Range 0 - 1600's (approximate)
Used to calculate cell density. This also indicates metabolic patterns. When Starved, OD plummets.

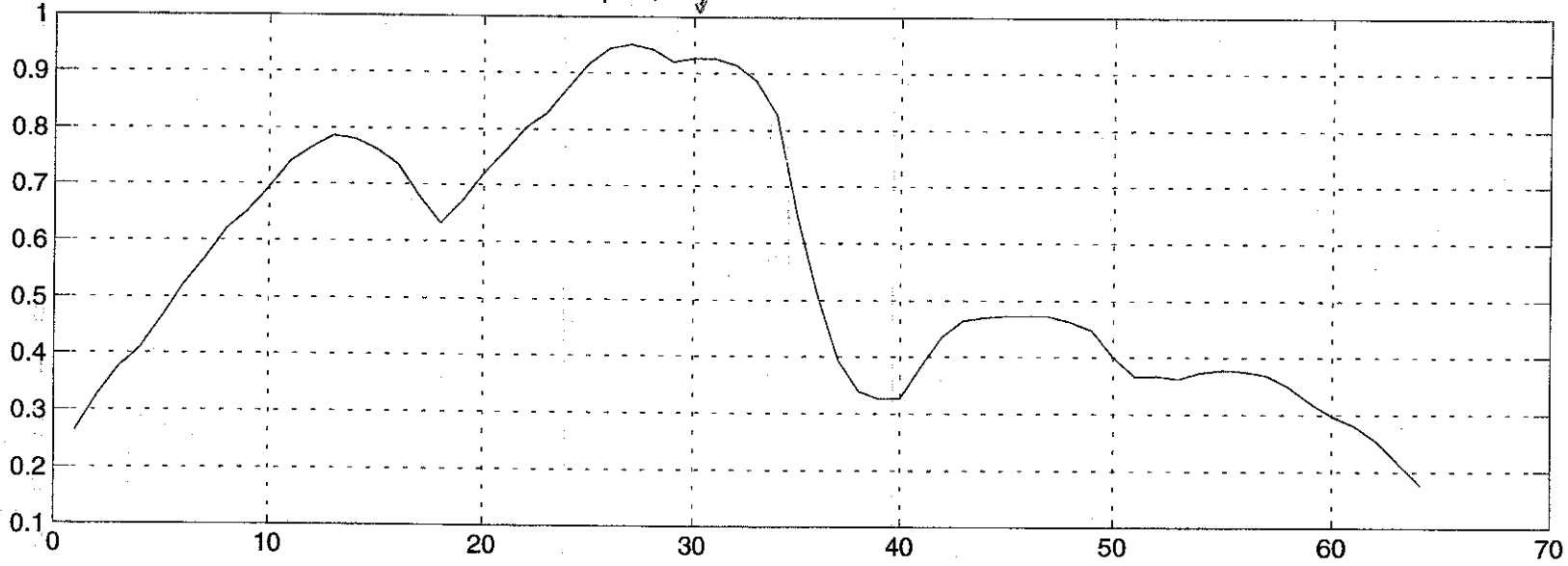
Glucose Conc: YSI 0 - ~1600 mg/dL 10 mg/dL Res.



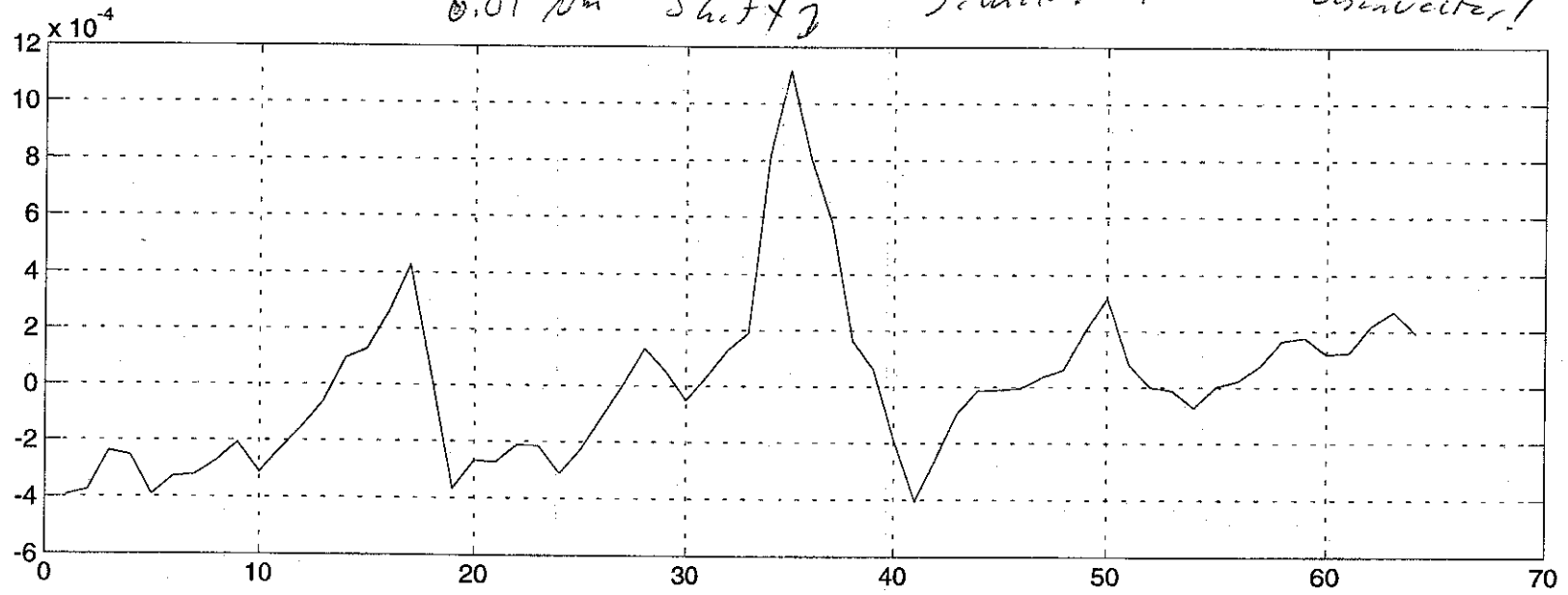
Best Western
Best Western Boulder Inn
770 28th Street
Boulder, CO 80303
(303) 449-3800
Fax: (303) 402-9118
For Reservations Call
1-800-233-8469

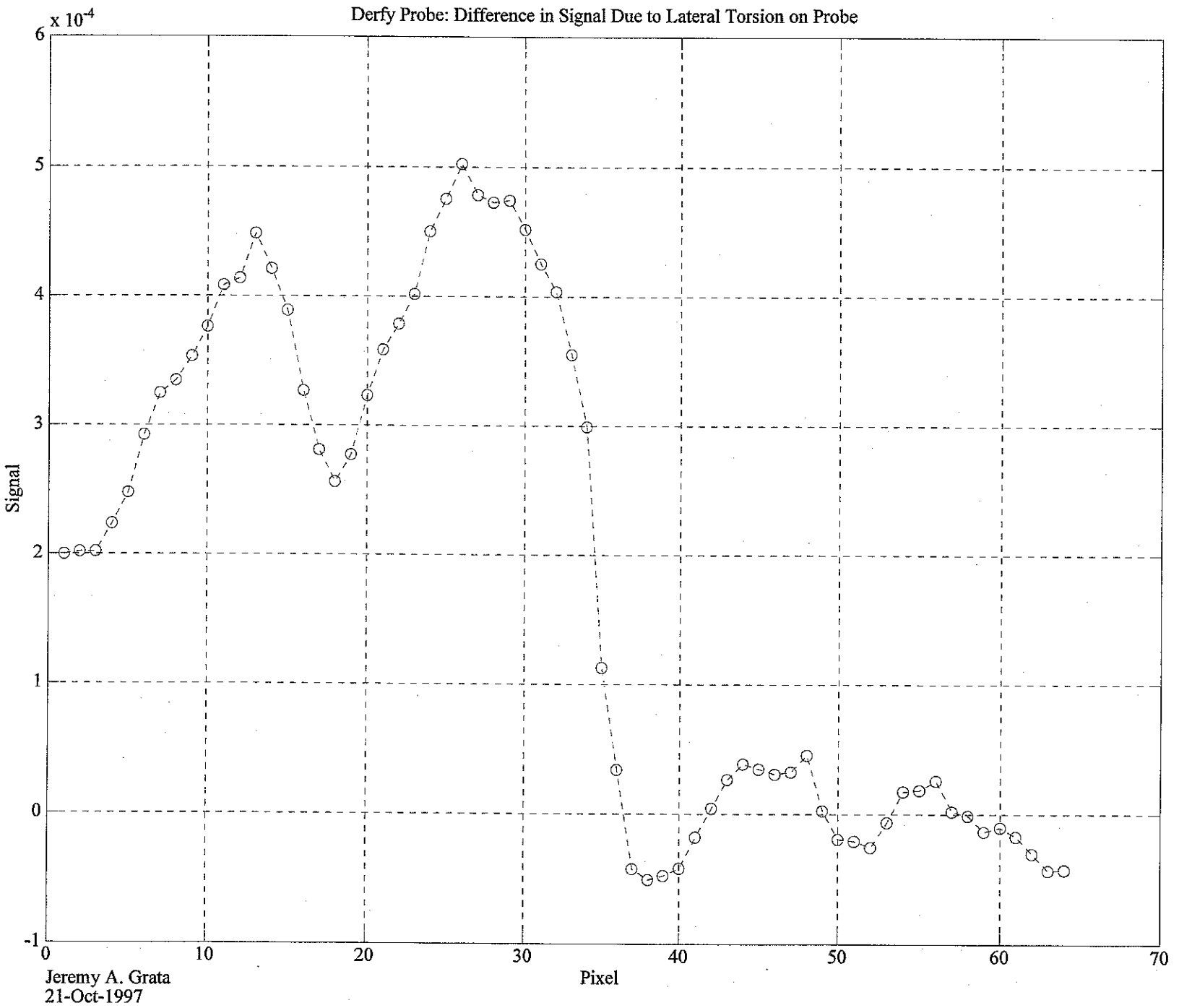
Jim Roland (Ryland?)
Director of all fermentor operations
Janette Larsen
In charge of prod. Dev.

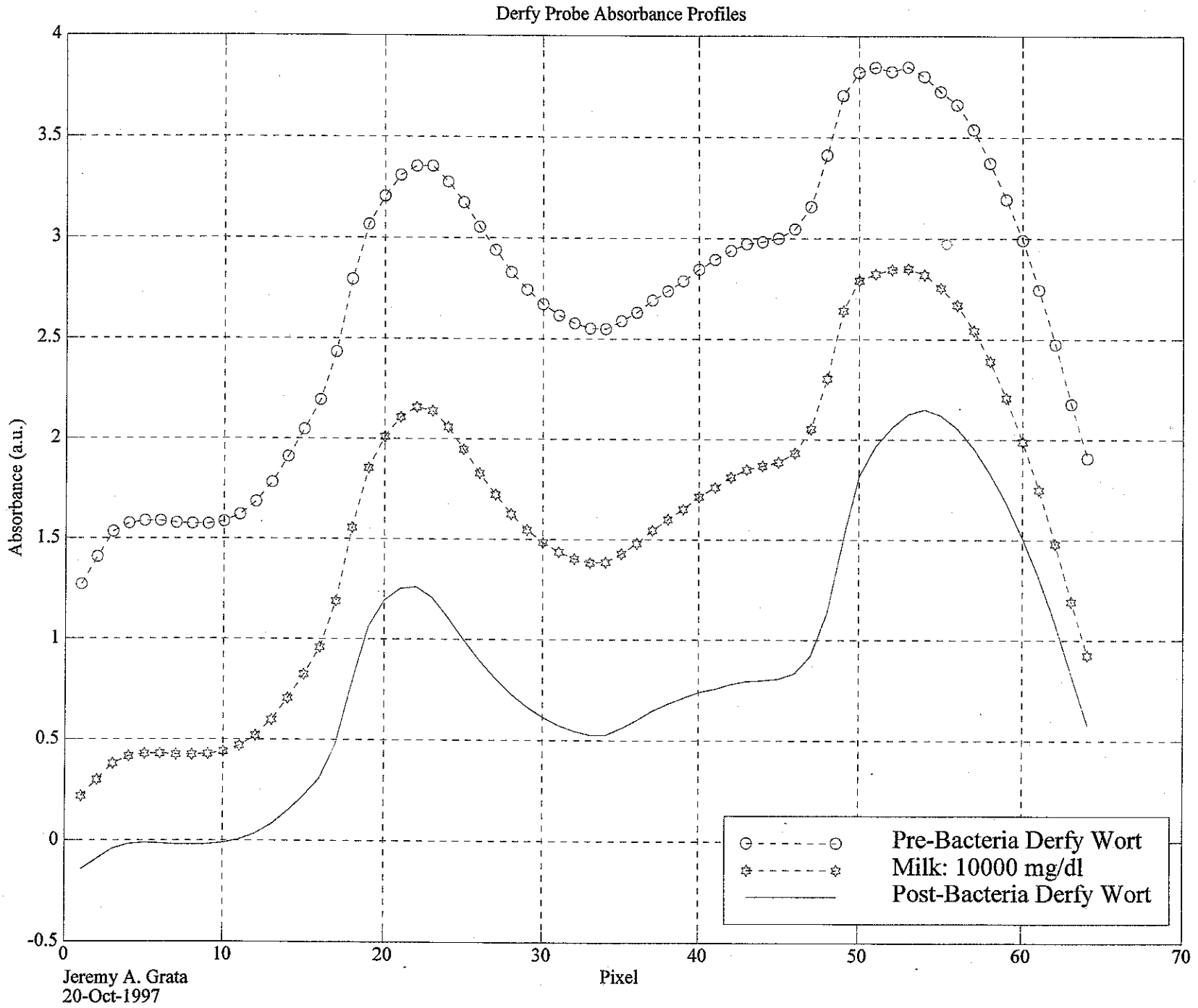
Ref 1

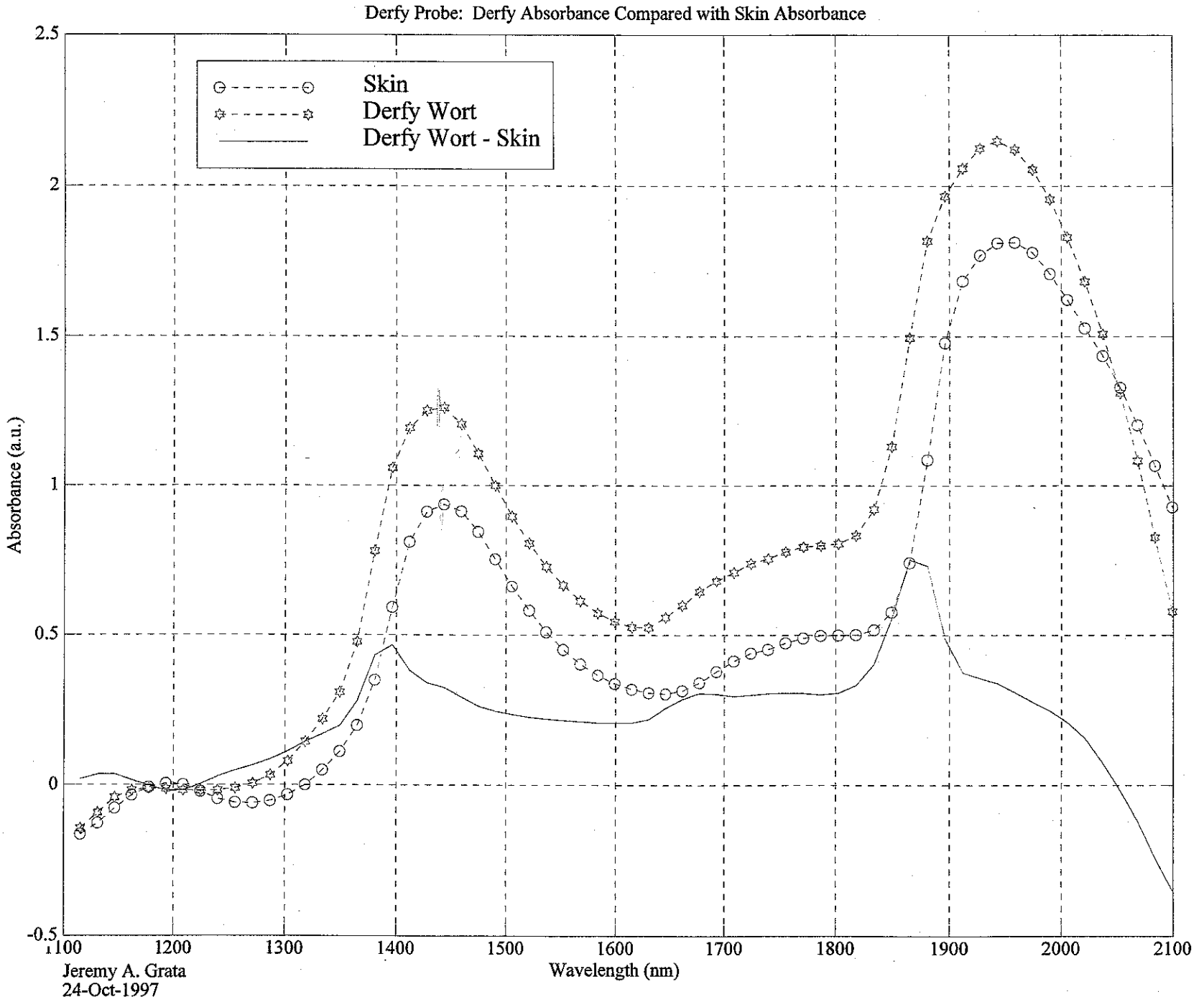


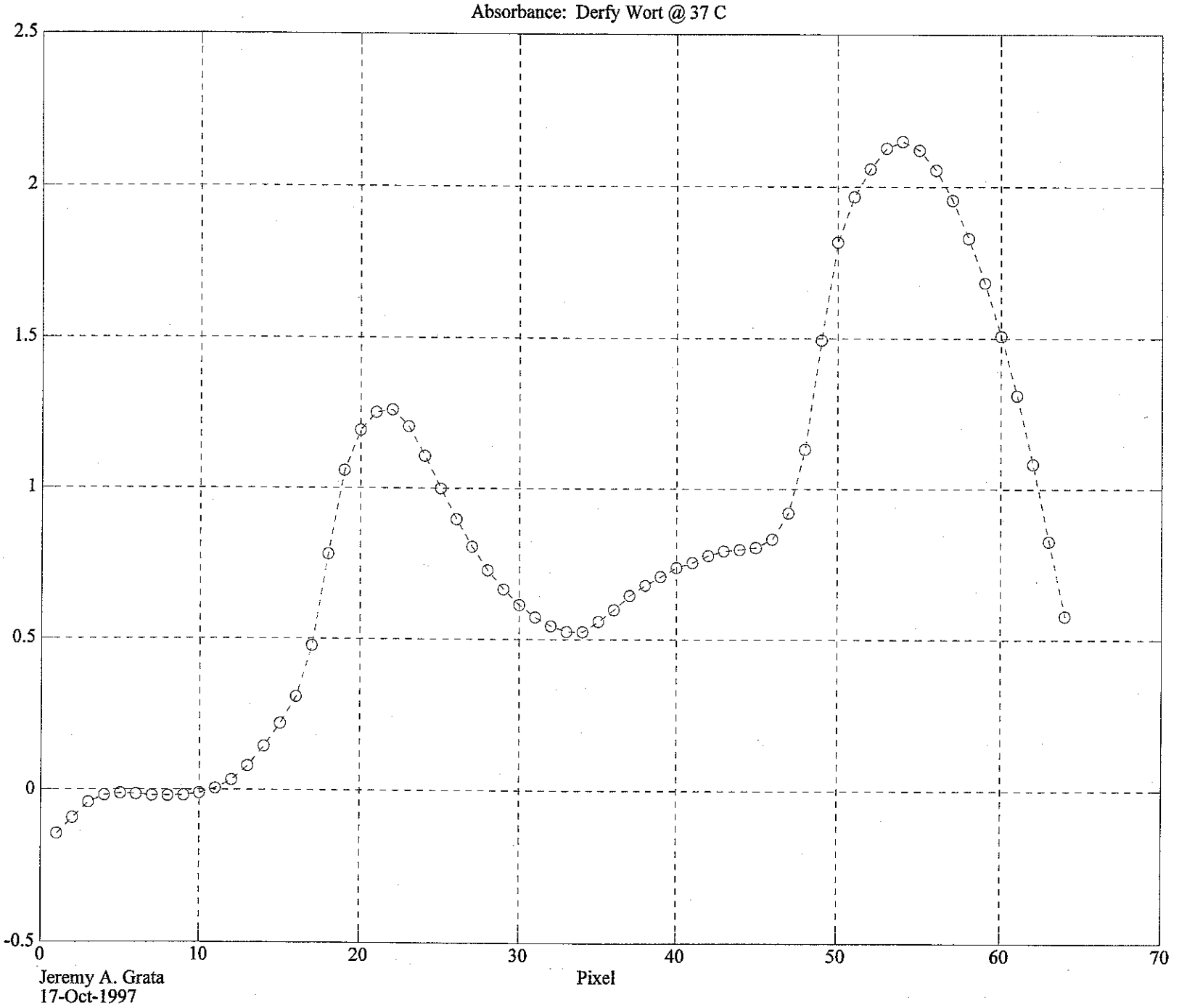
0.01 nm Shift 1 Similar to 1ST Eigenvector!

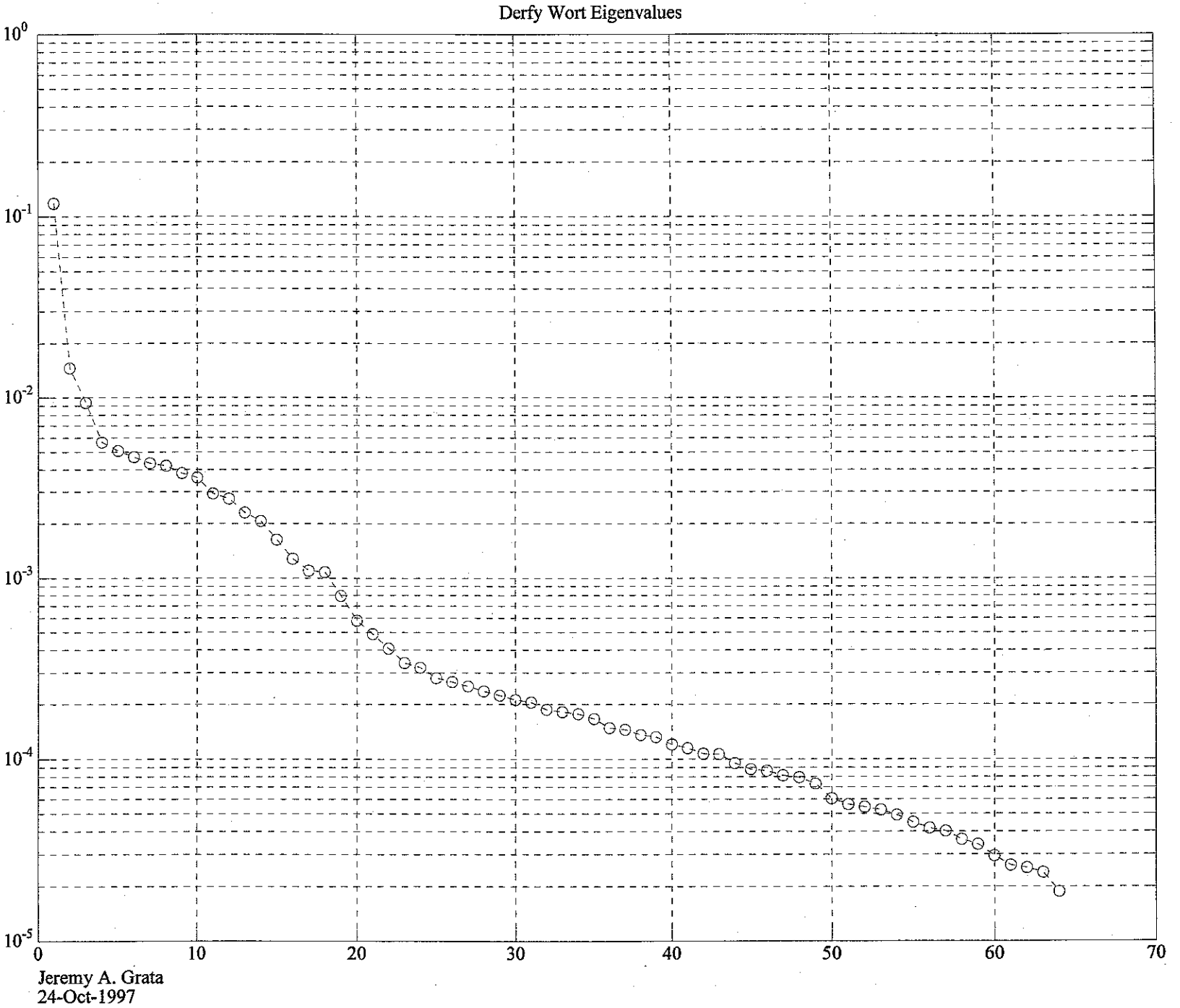


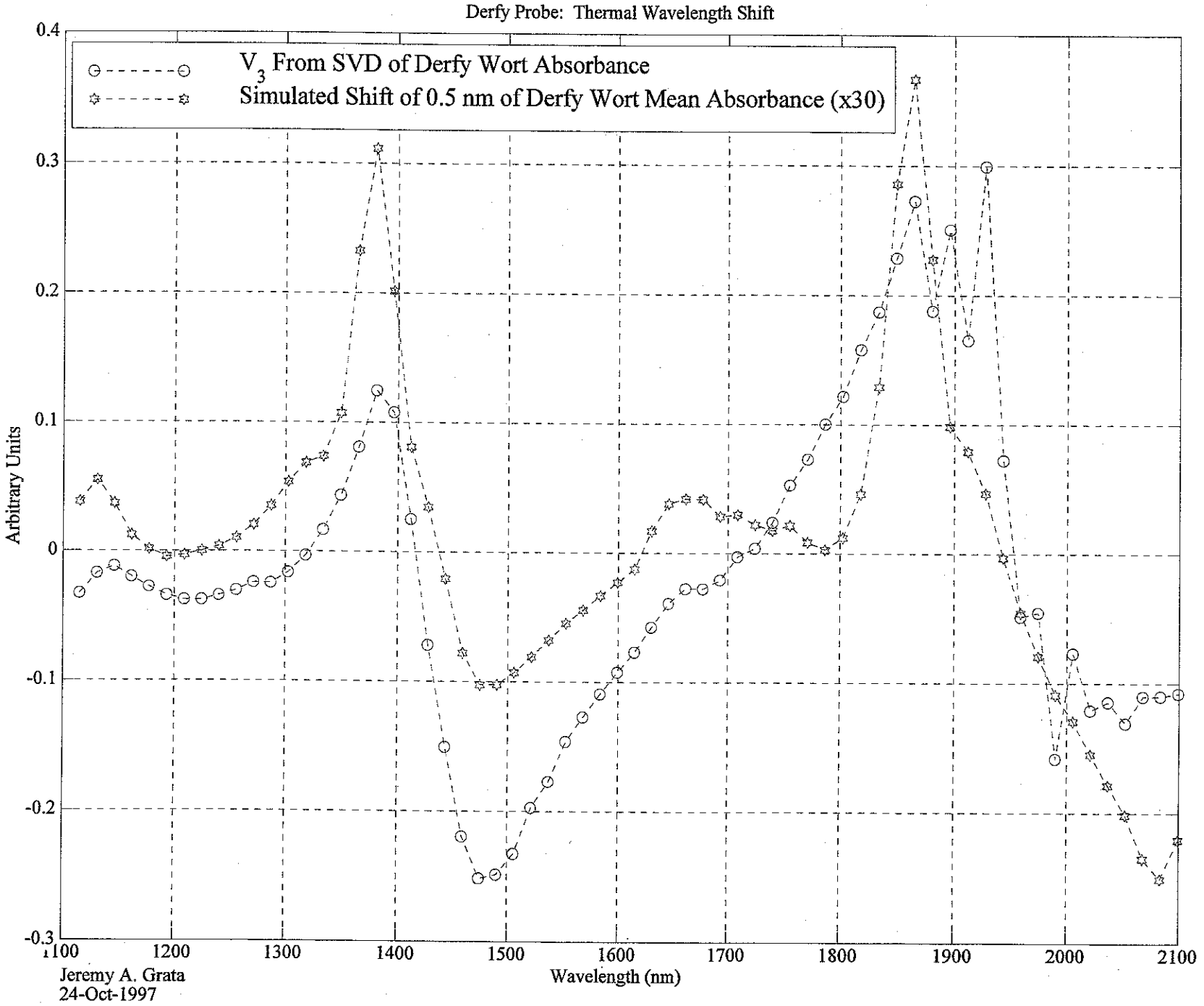

Derfy Probe: Difference in Signal Due to Lateral Torsion on Probe
x 10^-4
6
5
4
3
2
1
0
-1
Signal
0
10
20
30
40
50
60
70
Pixel
Jeremy A. Grata
21-Oct-1997

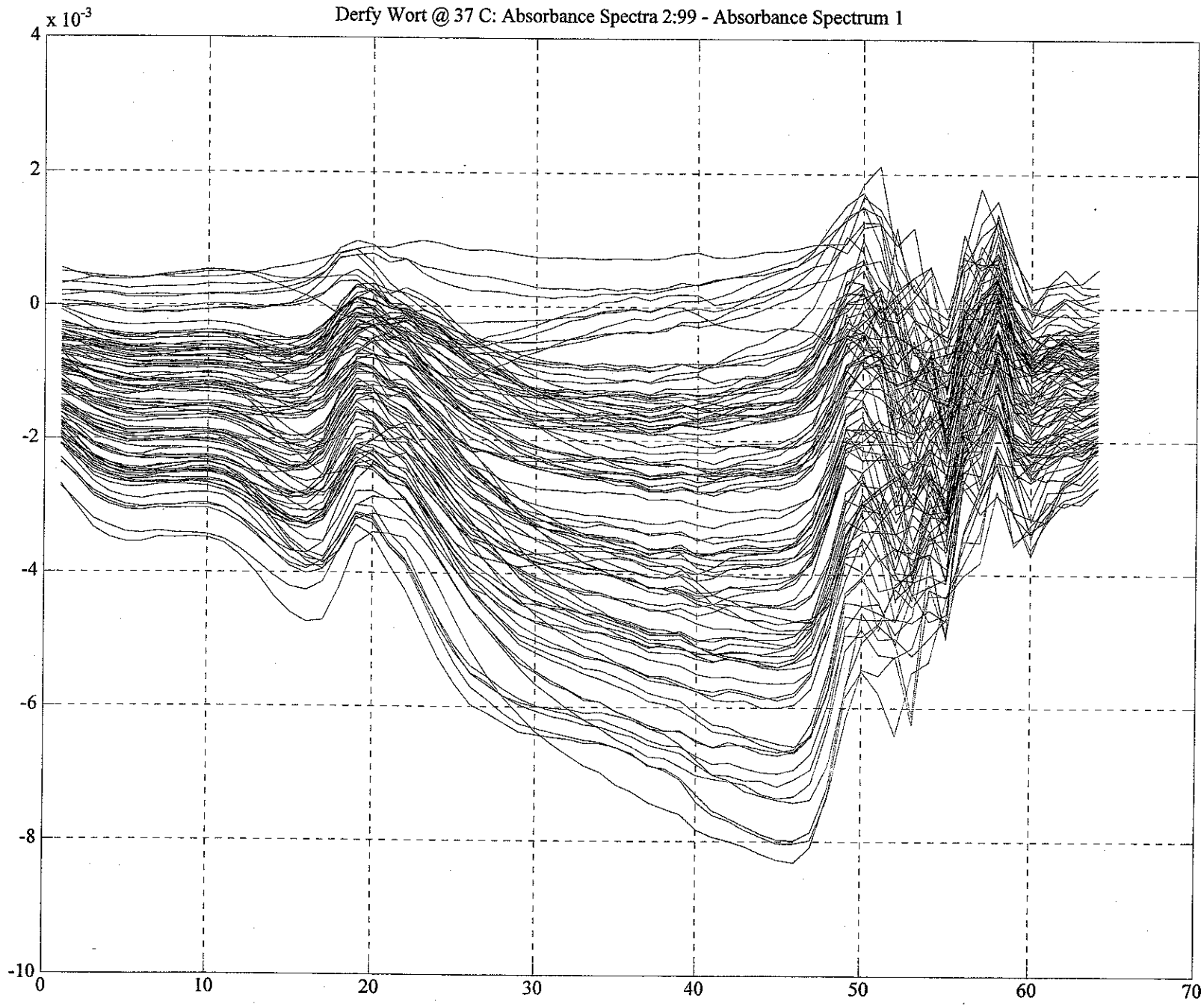

Derfy Probe Absorbance Profiles
Absorbance (a.u.)
Pixel
Jeremy A. Grata
20-Oct-1997
Pre-Bacteria Derfy Wort
Milk: 10000 mg/dl
Post-Bacteria Derfy Wort


Derfy Probe: Derfy Absorbance Compared with Skin Absorbance
Skin
Derfy Wort
Derfy Wort - Skin
Absorbance (a.u.)
Wavelength (nm)
2.5
2
1.5
1
0.5
0
-0.5
1100
1200
1300
1400
1500
1600
1700
1800
1900
2000
2100
Jeremy A. Grata
24-Oct-1997


Absorbance: Derfy Wort @ 37 C
2.5
2
1.5
1
0.5
0
-0.5
0
10
20
30
40
50
60
70
Pixel
Jeremy A. Grata
17-Oct-1997


Derfy Wort Eigenvalues
10^0
10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5}
0
10
20
30
40
50
60
70
Jeremy A. Grata
24-Oct-1997


Derfy Probe: Thermal Wavelength Shift
V3 From SVD of Derfy Wort Absorbance
Simulated Shift of 0.5 nm of Derfy Wort Mean Absorbance (x30)
Arbitrary Units
Wavelength (nm)
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
1100
1200
1300
1400
1500
1600
1700
1800
1900
2000
2100
Jeremy A. Grata
24-Oct-1997


Derfy Wort @ 37 C: Absorbance Spectra 2:99 - Absorbance Spectrum 1
x 10^-3
4
2
0
-2
-4
-6
-8
-10
0
10
20
30
40
50
60
70

BIOCONTROL TECHNOLOGY, INC.

Memorandum

TO: Jeremy Grata
FROM: Todd Barker
DATE: November 6, 1997
RE: **Experimental Design, BIOFERM**

In this memo I've prepared a randomized experimental design. The worksheet below shows a replicated and randomized design with Spike as a quantitative variable and Block as a qualitative variable. In the work sheet Blank simply indicates where nonglucose DI water is added to the Wort.

original design order	Sample #	Wort Concentration (mg/dL)	Block
11	1	350 400	Glucose
6	2	100	Blank
34	3	250 200	Glucose
33	4	100	Glucose
30	5	300	Blank
27	6	350 400	Glucose
40	7	500	Blank
9	8	100	Glucose
22	9	300	Blank
7	10	300	Blank
19	11	300 400	Glucose
28	17	500	Glucose
31	13	500	Blank
4	14	500	Glucose
8	15	300	Blank
17	16	100	Glucose
38	17	500	Blank
12	18	500	Glucose
13	19	300	Blank
3	20	350 400	Glucose
5		100	Blank
18		250 200	Glucose
16		500	Blank
1		100	Glucose
39		300	Blank
25		100	Glucose
23		100	Blank
26		250 200	Glucose
24		100	Blank
35		350 400	Glucose
15		100	Blank
21		100	Blank
14		300	Blank
37		500	Blank
2		250 200	Glucose
29		300	Blank
36		500	Glucose
10		250 200	Glucose
20		500	Glucose
32		500	Blank

10:46

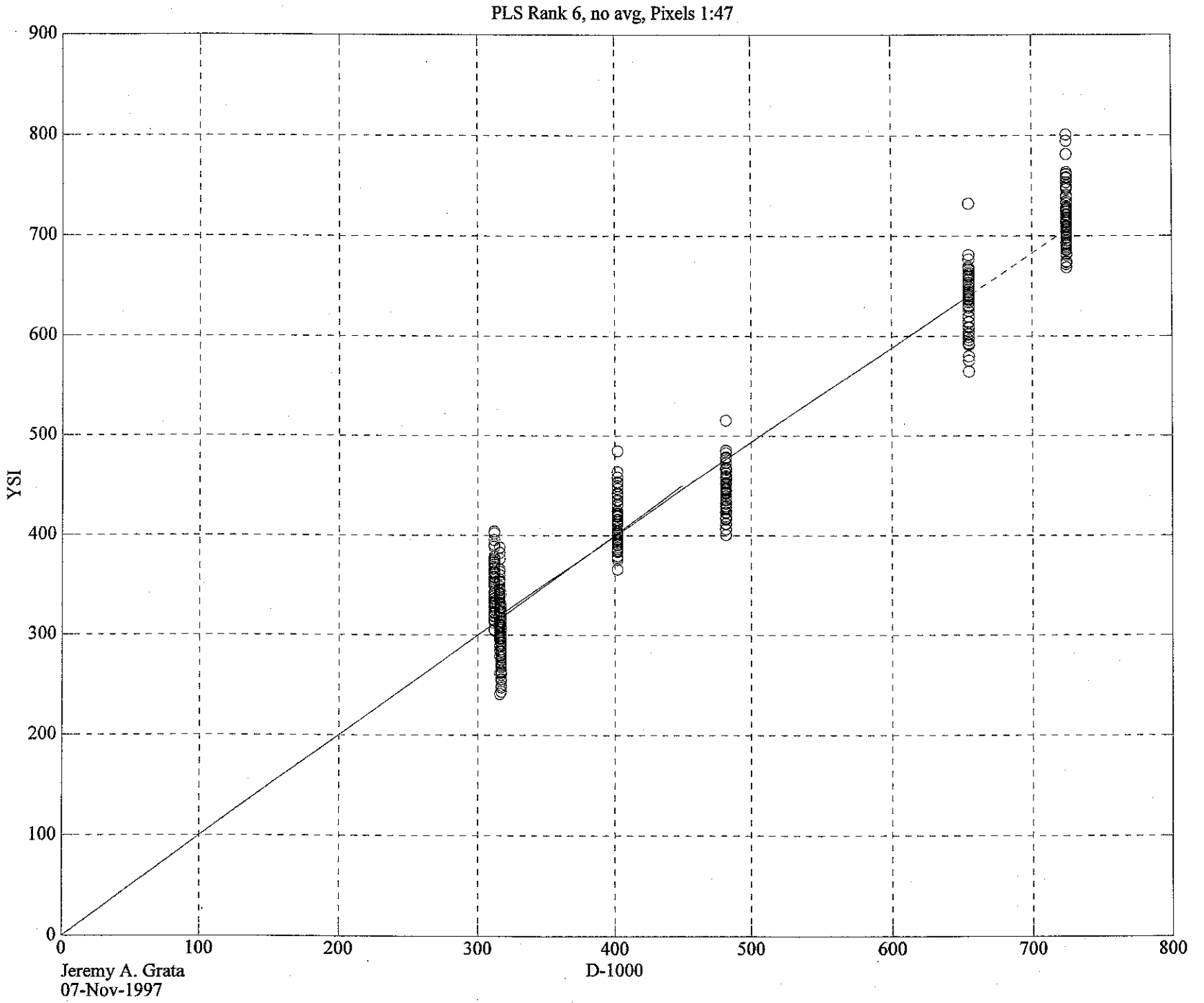
11:12

We might also consider more extensive replication. I've also put together a design having 10 replicates and two series of dilutions - perhaps allowing for more effective control of pipet uncertainty or variation due to time correlation.

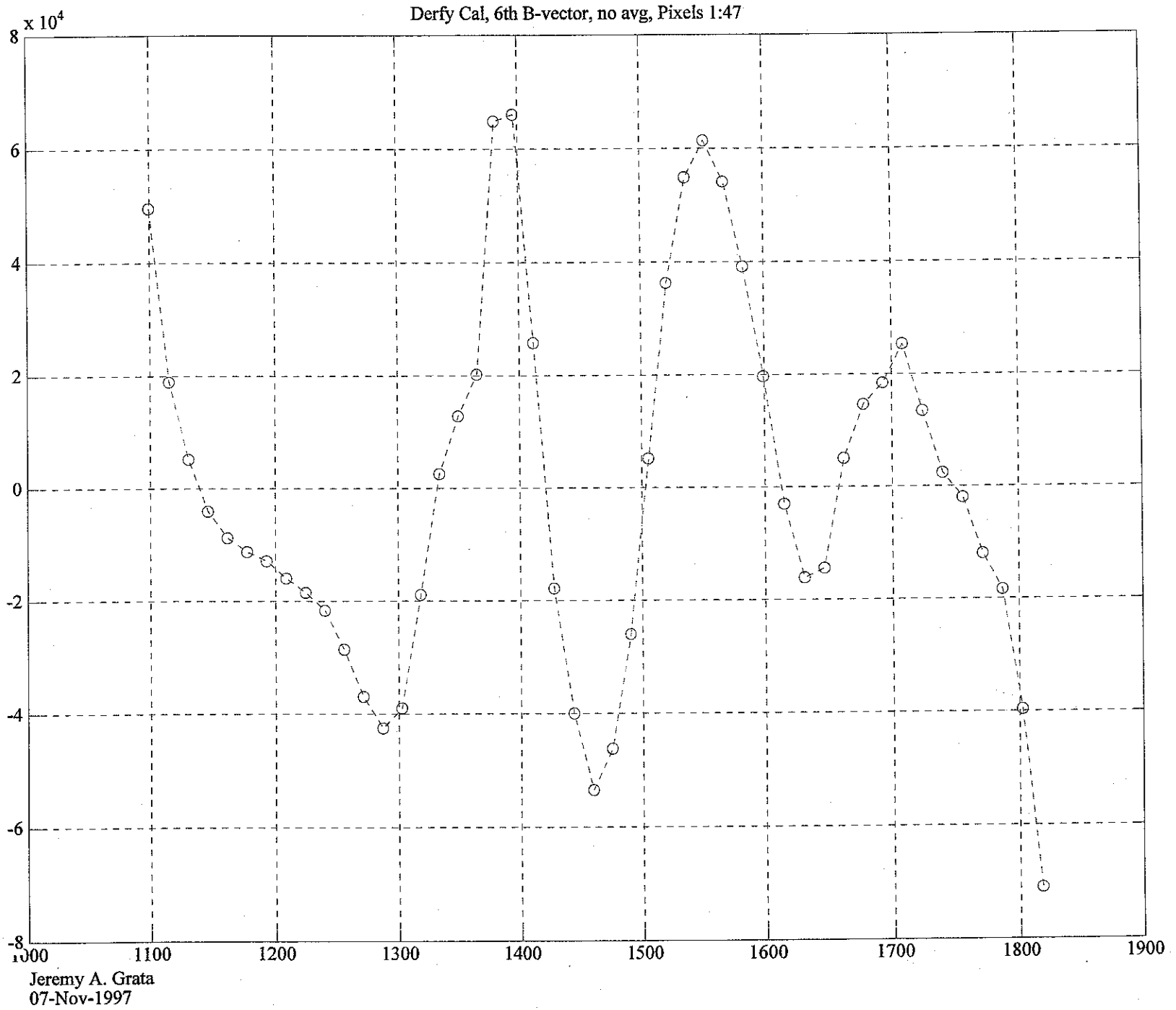
The following solutions might work well - apparently you've not found the solubility of glucose in water to be an issue - which is good. Hopefully we can run through the 40 tests on Friday.

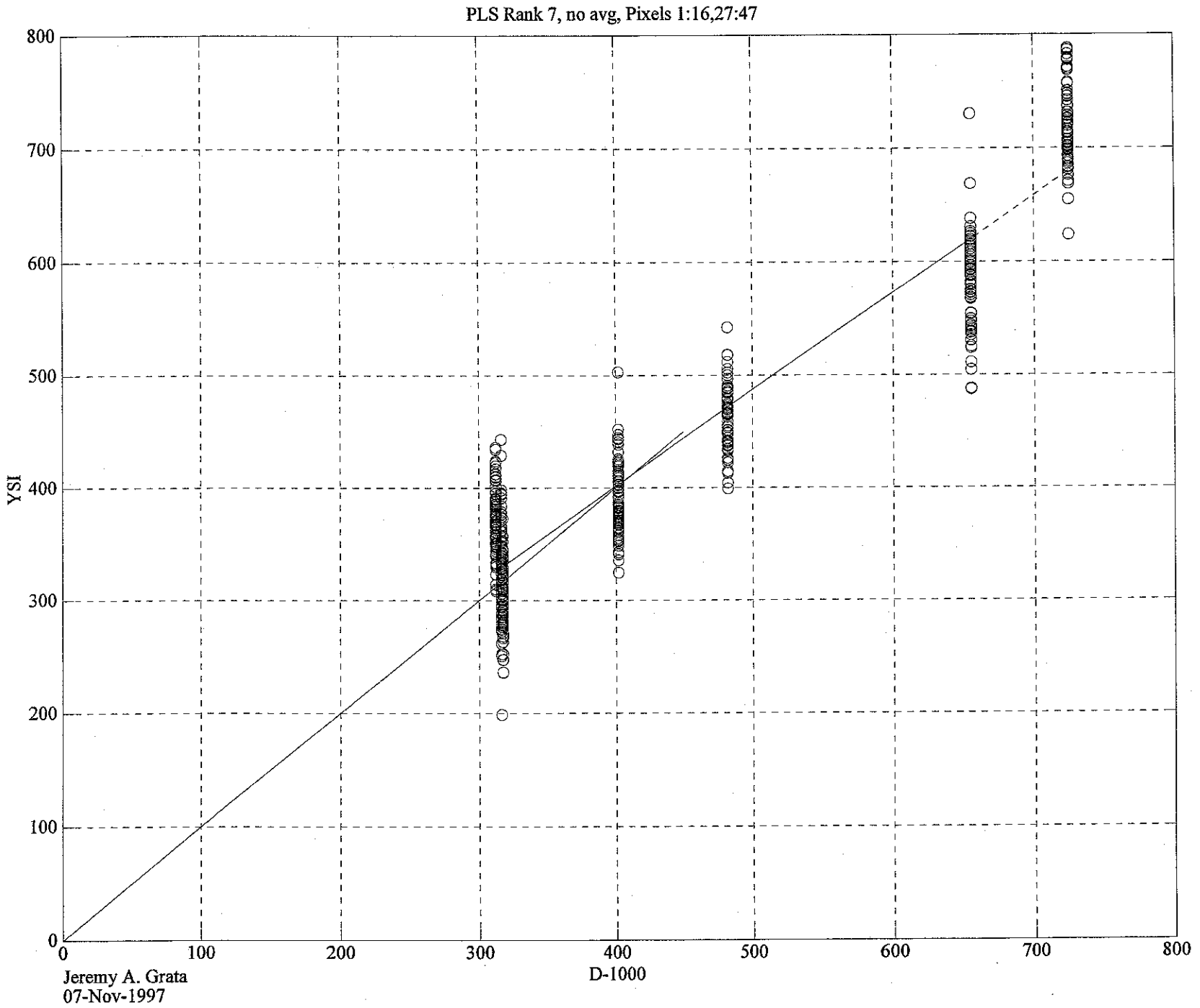
Prepare spiking solution to a concentration of 500 g / L (50,000 mg/dL). This is approximately 2:1 volume/volume solution and may require heating or ultrasonic dissolution.

BIOFERM Wort Solution Concentration	Dilution Scheme Spike Volume / Wort Volume	Stock Concentration Spiking Solution
100	250 µL Stock / 125 mL	50000 mg/dL
~~200~~ 250	~~625~~ µL Stock / 125 mL	50000
300	750 µL Stock / 125 mL	50000
~~400~~ 350	~~875~~ µL Stock / 125 mL	50000
500	1250 µL Stock / 125 mL	50000


PLS Rank 6, no avg, Pixels 1:47
YSI
D-1000
Jeremy A. Grata
07-Nov-1997
0
100
200
300
400
500
600
700
800
900

1:16 27:47


Derfy Cal, 6th B-vector, no avg, Pixels 1:47
x 10^4
8
6
4
2
0
-2
-4
-6
-8
1000
1100
1200
1300
1400
1500
1600
1700
1800
1900
Jeremy A. Grata
07-Nov-1997


PLS Rank 7, no avg, Pixels 1:16,27:47
YSI
D-1000
0
100
200
300
400
500
600
700
800
Jeremy A. Grata
07-Nov-1997

WORT
51.0 ml 51.6 grams 129 grams 125 ml

Experimental Design — See 11-6-97

① Stock Solution Prepared by Jeremy Grota 50,000 mg/dL

Nominal	WORT Conc	Dilution	YSI reading
1	100	250 ul / 125 ml	410 mg/dL
2	200	500 ul / 125 ml	491 mg/dL
3	400	1000 ul / 125 ml	655 mg/dL
4	500	1250 ul / 125 ml	750 mg/dL

② Stirred thermostatted soln 37°C
Sample pipet for YSI measu[...]t [...] drawn from
[...]ed soln.

YSI rea[...]

③ Blanks

5	100
6	300
7	500

④ ~~50~~ 75 Refere[...]
~~50~~ 75 Dark[...]
~~100~~ 75 Spec[...] [...]ck

655	mg/dL 4[...]
318	1[...]
491	2[...]
410	1[...]
	~~DRK~~ REF
	~~REF~~ DRK
315	30[...]
655	400
318	500
410	100
	REF
	DRK

R ~~80~~ 75
D ~~60~~
S ~~100~~

R D 11:36
R D 12:28
R D 11:57 S 41.5
R D 11:55
R D 12:44
D 236
500 34.5
300B 34
100 37.5
~~500~~ B 34
400 35
200 36
100 B 38
R D 1:20
R D 12:10
S 42.5 S
R D 3:01

Signed

PROJECT BIOFERM

Continued From Page 10

YSI	Nominal	Type	Stir 5 ↓	Temp	Time Clock
315	300	B		38.0	
315	300	B		38.0	
655	400 ~~300~~	G		38.5	
750	500	G		42.5	
	REF				
	DRK				
318	500	B		40.5	
750	500	G		41.5	
318	500	B		400	
410	100	G		39.5	
	REF				
	DRK				11:53
318	500	B		38.5	
750	500	G		41.5	
315	300	B		44.0	
655	400	G		41.0	
	REF				
	DRK				12:10
318	100	B		41.5	12:13
491	200	G		47.0	
318	500	B		41.0	
410	100	G		41.0	
	REF				
	DRK				12:28
315	300	B		41.5	
410	100	G		39.5	
318	100	B		43.5	
655	400	G		41.0	
	REF				
	DRK				12:44

Continued on Page

Read and Understood By

Signed | Date | Signed | Date

PROJECT

YSI	Nominal	Type	Stir	Temp	Time Clock
318	100	B		41.5	
318	100	B		41.0	
315	300	B		41.0	
318	500	B		42.5	
	R				
	DRK				
491	200	G		44.0	
315	300	B		44.0	
750	500	G		45.5	
491	200	G		43.0	
	REF				1:16
	DRK				
750	500	G		43	
	500	B		41.5	1:23

Continued on Page

Read and Understood By

Signed	Date	Signed	Date

11-11-97

Derby Validation Session

10:24	75 Ref		No dark correction		
10:25	75 Dark		"		
10:26	75:52	24°C	"	Star @ 6	.500
					497
10:29	75:56	24.5°C	"	"	322
					318
10:32	75:55	24.5°C	"	"	321
					319
10:35	75:51	24.5°C	"	"	405
					407
10:38	75 Ref		"		
10:39	75 Dark		"		
10:40	75:54	25.5°C	"	"	758
					758
10:44	75:57	24.5°C	"	"	318
					—
10:46	75:53	24°C	"	"	.673
					670
10:49	75 Ref		"		
10:50	75 Dark		"		

YSI BLOOD GLUCOSE RESULTS

11/07 11/11

PATIENT ID CODE #	PHLEB TIME	YSI (1)	TIME ~~YSI (2)~~	YSI 1 ~~OPERATOR~~	YSI 2 ~~REMARKS~~
000 Sample 1	10:10	410	10:30	405	407
2	10:12	491	10:35	500	497
3	10:19	655	10:39	673	670
4	10:21	750	10:43	758	758
5	10:23	318	10:47	321	319
6	10:26	315	10:51	322	318
7	10:28	318	10:55	328 injected pkg.	318
1	2:29	402			
2	2:31	482			
3	2:33	655			
4	2:35	725			
5	2:38	316			
6	2:40	317			
7	2:42	312			

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Index of Files Recorded on Derfy Probe Machine

Repotl - Repositioning the reference by tightening and loosening the reference holder. 20 with bolt just snug, 20 with bolt tightened, 120 total.

Reposref - Repositioning the reference holder by removing and replacing. 100 Dark, 100 Reference (w/o removing the reference). 2 Sets of 20 Repositioned references, 5 individual references (one repositioning), 4 sets of 20 Repositioned references

Wiggle - The body of the probe is subjected to torsion in different directions to see the effect of probe vibration. 100 Dark, 300 Reference spectra non-stop. First two peaks of the reference spectra are due to lateral torsion on probe (towards back first then towards front. This assumes the probe is hanging off to one side of the d1000, the start button side). These two torsion applications are repeated, then a sequence of torsion applications follows in this manner: towards the back, towards the front, away from the d1000, towards the d1000 (same probe orientation as above). This sequence is performed 5 times, then 2 sets of towards back/towards front torsion applications.

Openwow - This is open probe data. Because of the platform that the d1000 is attached to, open-probe signal must be taken either from the platform or from a reflecting surface. 100 dark, 100 Spectra of black felt on the probe facing surface of the platform, 100 dark, 100 spectra from a front surface gold reflecting mirror positioned under the probe at roughly a 45 degree angle from the probe output light direction.

Milk20k - 20000 mg/dl milk solution spectra recorded under the typical derfy probe sampling method. 100 Dark, 100 reference, 100 milk spectra in continuously stirring bath @ 35-37 degree C inside a 300ml glass dish sitting on a piece of aluminum oxide, 100 milk (same as above, but with a piece of krylon black painted note card between the surface the aluminum oxide and the bottom of the dish.

Lastdrd - These are reference spectra for noise analysis. The machine was allowed to warm up for a few days. 200 dark spectra, 600 reference spectra, 200 dark spectra.

Derfwatr - Water spectra recorded under the typical derfy probe sampling method.

Milkwarm - 10000mg/dl milk solution recorded as above.

Derfpre - Derfy pre-bacteria solution recorded under the typical derfy probe sampling method.

Derfp - Derfy harvested bacteria solution (wort) recorded under the typical derfy probe sampling method but with one extra reference recorded after the normal 100 references and at room temperature.

Derfwarm - Derfy Harvested bacteria solution (wort) recorded under the typical derfy probe sampling method.

{**Derfdark, Derfdrk, Derfdrk2, Derfrd, Derfref, Derfref3**}- These file are the first sets of data recorded on the derfy machine. They contain refs, darks or both. For files with both, it can be determined if dark correction was on or off depending on the magnitude of the darks (~1e-3 if off, ~1e-5 if on). The same is true for files with dark only. For files with reference only, it is unknown if the dark correction was on or off. Some of these files were recorded before the probe was completely wrapped in insulation. For analysis of dark and reference, use **Lastdrd**.