

# Hemox Analyzer Instructions

Prepared by Nik Gourianov

# Site worth knowing



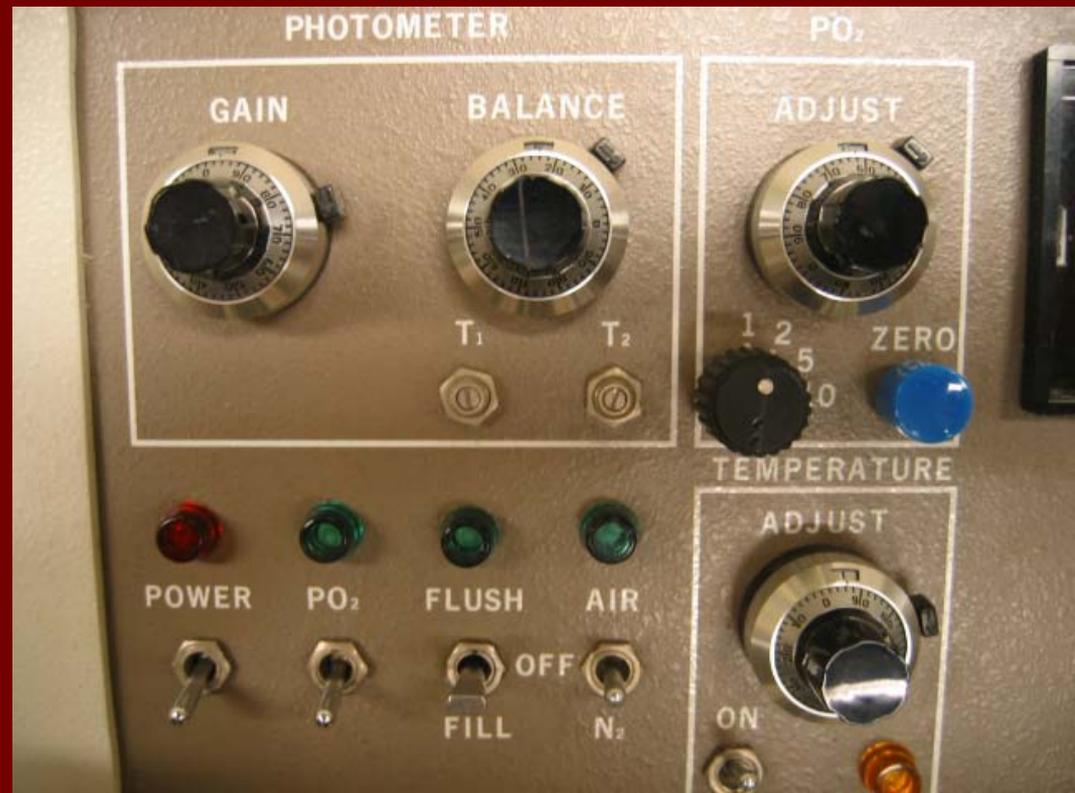
- Never know what you can find on labx.com
- One man's junk is other man's treasure...

# Hemox Manual

- Install oxygen probe membrane (usually done a day or two before)
- Oxygenate your sample for 2 hours under  $O_2$  flow at  $0\text{ }^\circ\text{C}$
- Dilute oxygenated sample to about 1 g/L of Hb concentration
- Turn  $N_2$  and Air gas flow on (approx. 10 PSI each)
- Turn water cooling/heating system on

# Hemox Manual

- Turn on POWER
- Fill the oxygenated Hb sample using 'FILL' switch
- Make sure there are no air bubble stuck against oxygen probe (gentle shaking and tilting should do)
- Turn  $PO_2$  switch on
- Turn AIR switch on



# Hemox Manual

- Adjust temperature control to match the temperature set on recirculation water bath
- Allow sample to equilibrate for 30-60 min.
- Orange light has to flash indicating that temperature has reached the setting



# Hemox Manual

- Switch to S1 and adjust reading to any value between 2.0 and 3.0 using GAIN knob
- Switch to S2 and obtain the same value using BALANCE knob
- Switch to S1/S2 and check if the value is very close to zero (usually less than 0.010 is desired)
- If not, repeat S2 balancing

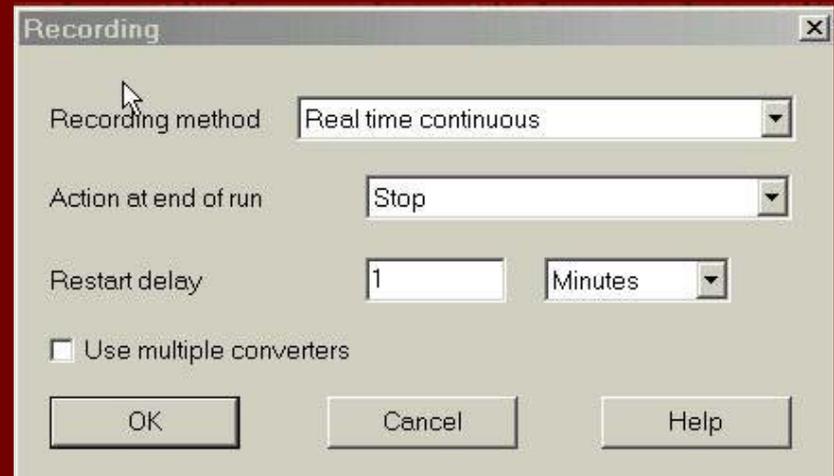


# Hemox Manual

- Switch to PO<sub>2</sub> reading and adjust with PO<sub>2</sub> knob to get 150 value
- The value should not change within 5 min time.
  - If it changes give more equilibration time and than adjust to 150
- Set/leave multiplier (small knob next to PO<sub>2</sub>) to position 1
- Open Pico Log Recorder software

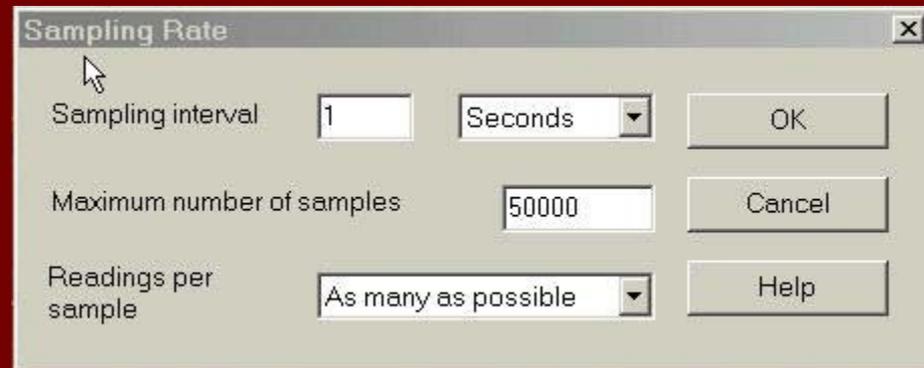
# Hemox Manual

- Click settings and choose recording tab
- Set/leave real time continuous method and stop action at the end of run
- Click OK



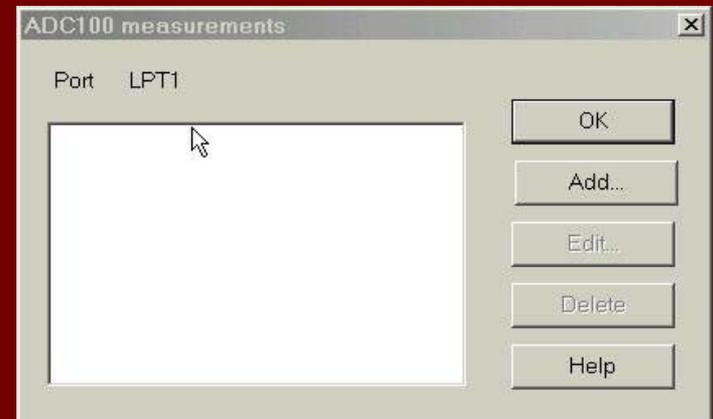
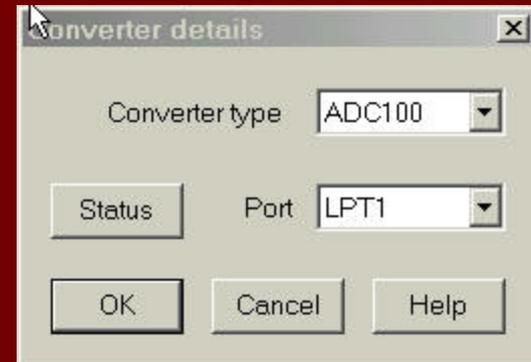
# Hemox Manual

- Click settings again and choose sampling rate
- Set/leave sampling interval to 1 second
- Set max number of samples to 50,000
- Leave reading per sample set at as many as possible
- Click OK



# Hemox Manual

- Click settings again and choose converter details
- Make sure you have ADC100 on LPT1 port
- Click OK
- This will take you to ADC100 measurements details window
- If window is empty, you will have to add channels by clicking Add...



# Hemox Manual

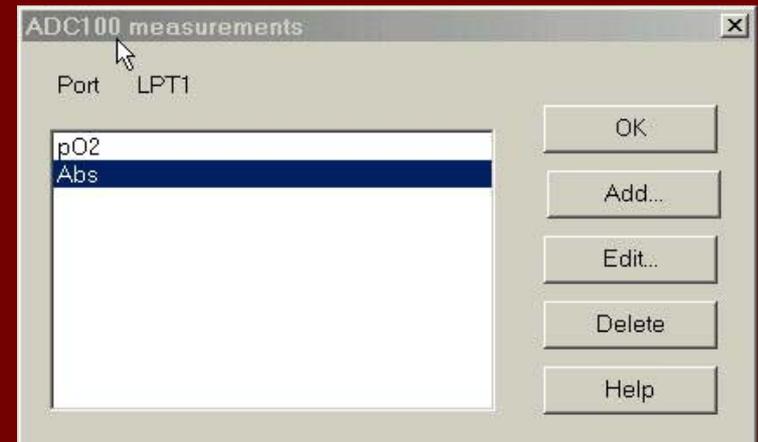
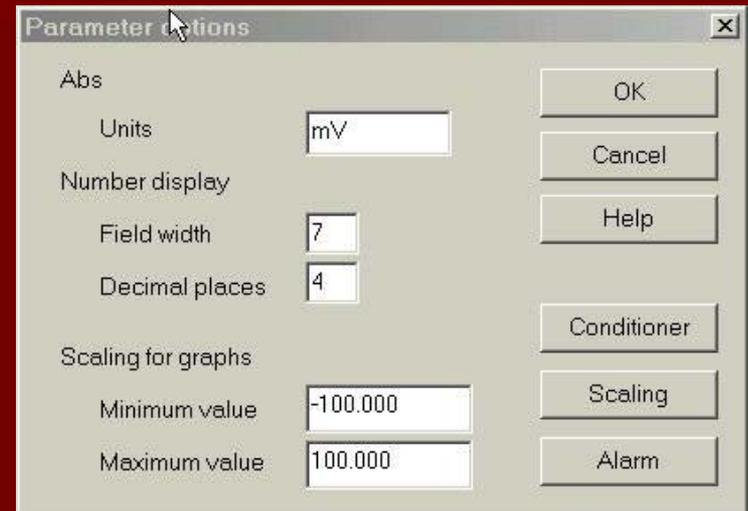
- Name first channel pO2 and set voltage range to +/-2V
- Click OK
- Add next channel and name it Abs
- Select channel B and choose voltage range +/-100mV
- Make sure measurements are set to DC volts on both channels

The screenshot shows the 'Edit ADC100 measurement' dialog box. The 'Name' field contains 'pO2'. The 'Channel' dropdown is set to 'Channel A'. The 'Measurement' dropdown is set to 'DC Volts'. The 'Voltage range' dropdown is set to '±2V'. The 'Scan time' field contains '50000' with 'us' to its right. On the right side, there are five buttons: 'OK', 'Cancel', 'Help', and 'Options...'.

The screenshot shows the 'Edit ADC100 measurement' dialog box. The 'Name' field contains 'Abs'. The 'Channel' dropdown is set to 'Channel B'. The 'Measurement' dropdown is set to 'DC Volts'. The 'Voltage range' dropdown is set to '±100mV'. The 'Scan time' field contains '50000' with 'us' to its right. On the right side, there are five buttons: 'OK', 'Cancel', 'Help', and 'Options...'.

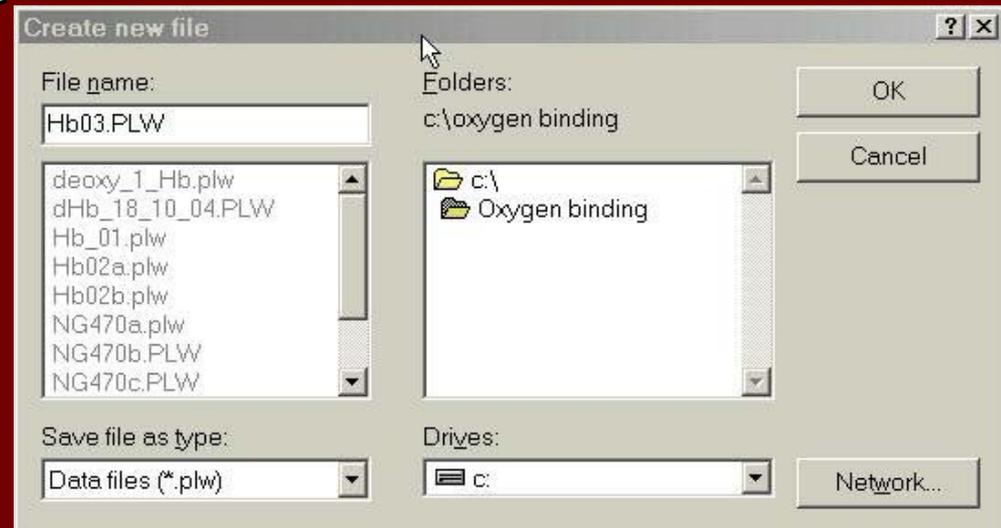
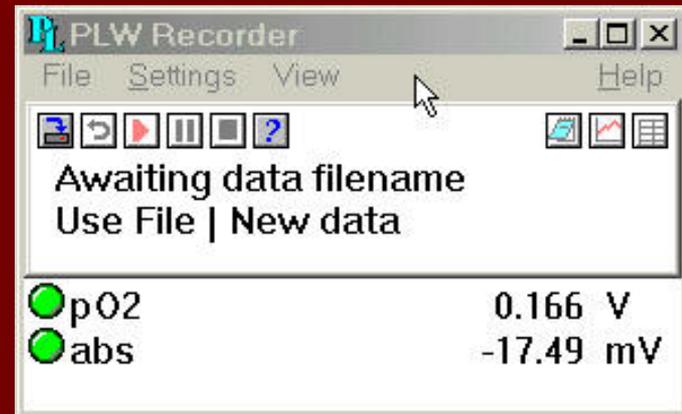
# Hemox Manual

- Click options and change number of decimal places to 4 (for both channels)
- Click OK
- Now you have both channels set up
- Click OK



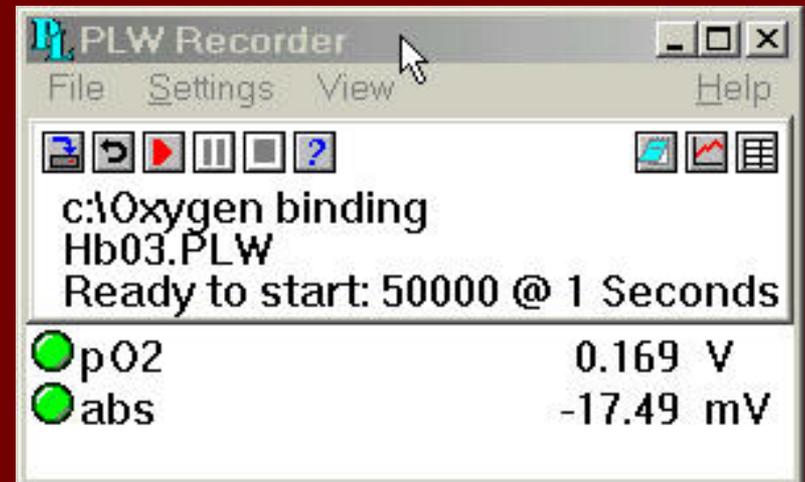
# Hemox Manual

- Click on New File tab and point to "Oxygen Binding" directory on C drive
- Create new file name under this directory (the file for your measurements)
- Click OK



# Hemox Manual

- Now record button is activated (red)
- When sample is equilibrated you can begin measurement by flipping gas flow to N<sub>2</sub> and clicking record button simultaneously
- Stop the run when pO<sub>2</sub> reading reaches 1 torr or less (0.01 V on software screen)



# Results

PLW Player

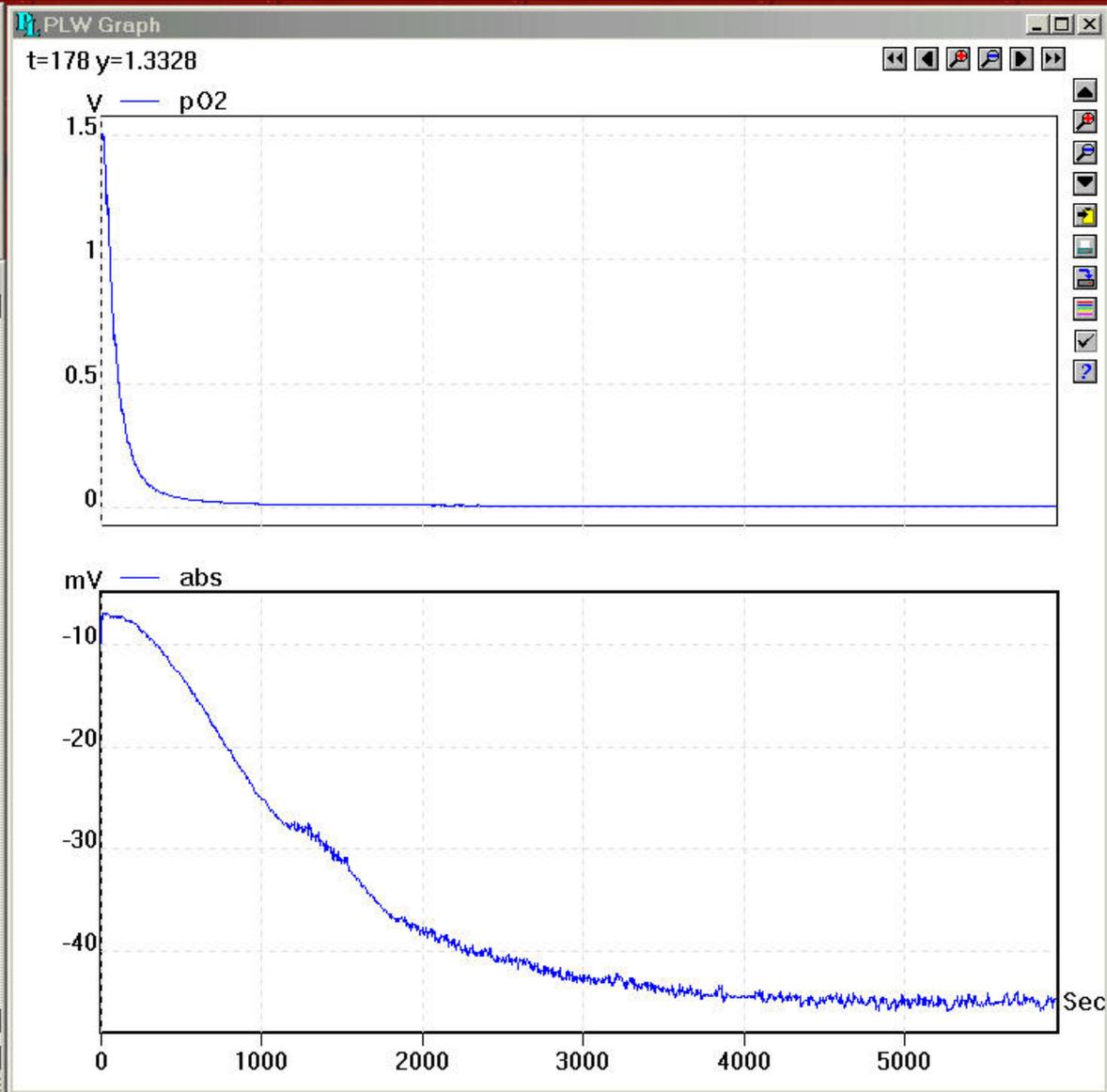
File Settings View Help

◀ ▶ ⏪ ⏩ ?

C:\Oxygen binding  
Hb02b.plw  
21/10/2004 15:36:28  
5942 samples

PLW Spreadsheet

Time	Seconds	pO2	abs
5831	0.0082	-44.8034	
5832	0.0084	-44.8705	
5833	0.0084	-44.5713	
5834	0.0083	-44.7728	
5835	0.0081	-44.8182	
5836	0.0082	-44.8644	
5837	0.0078	-44.9275	
5838	0.0082	-45.0476	
5839	0.0081	-44.9988	
5840	0.0082	-44.9316	
5841	0.0079	-45.2308	
5842	0.0084	-45.1453	
5843	0.0083	-45.0537	
5844	0.0083	-45.2003	
5845	0.0082	-45.2997	
5846	0.0085	-45.4106	
5847	0.0085	-45.4393	
5848	0.0085	-45.4703	
5849	0.0087	-45.4882	
5850	0.0087	-45.5178	
5851	0.0088	-45.6208	
5852	0.0088	-45.6827	



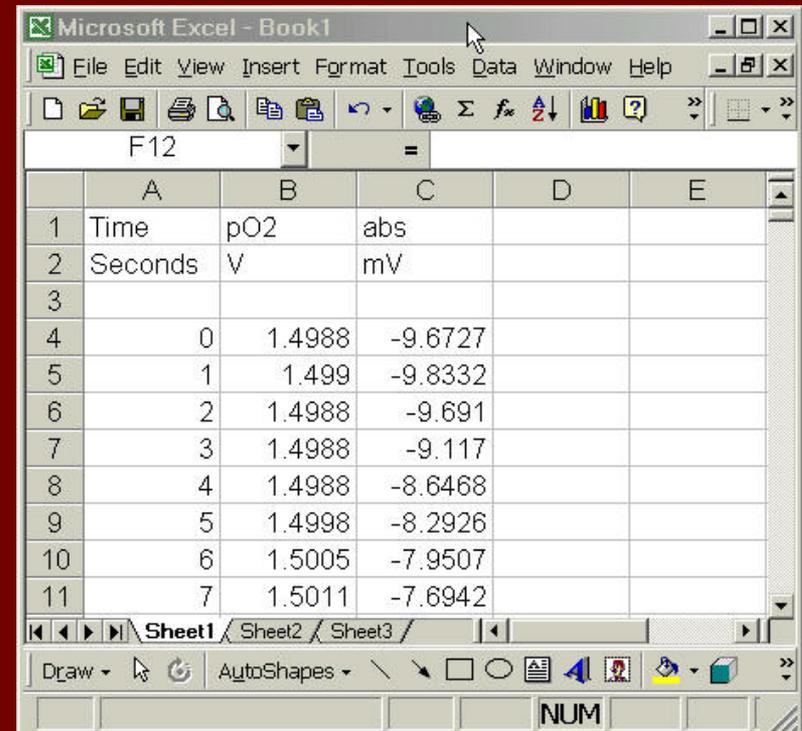
# Getting Data

- Highlight the collected data in the spreadsheet window
- Click copy to clip board tab
- Open EXCEL software and paste to it then Save it as an EXCEL file



PLW Spreadsheet

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5831	0.0082	-44.8034	
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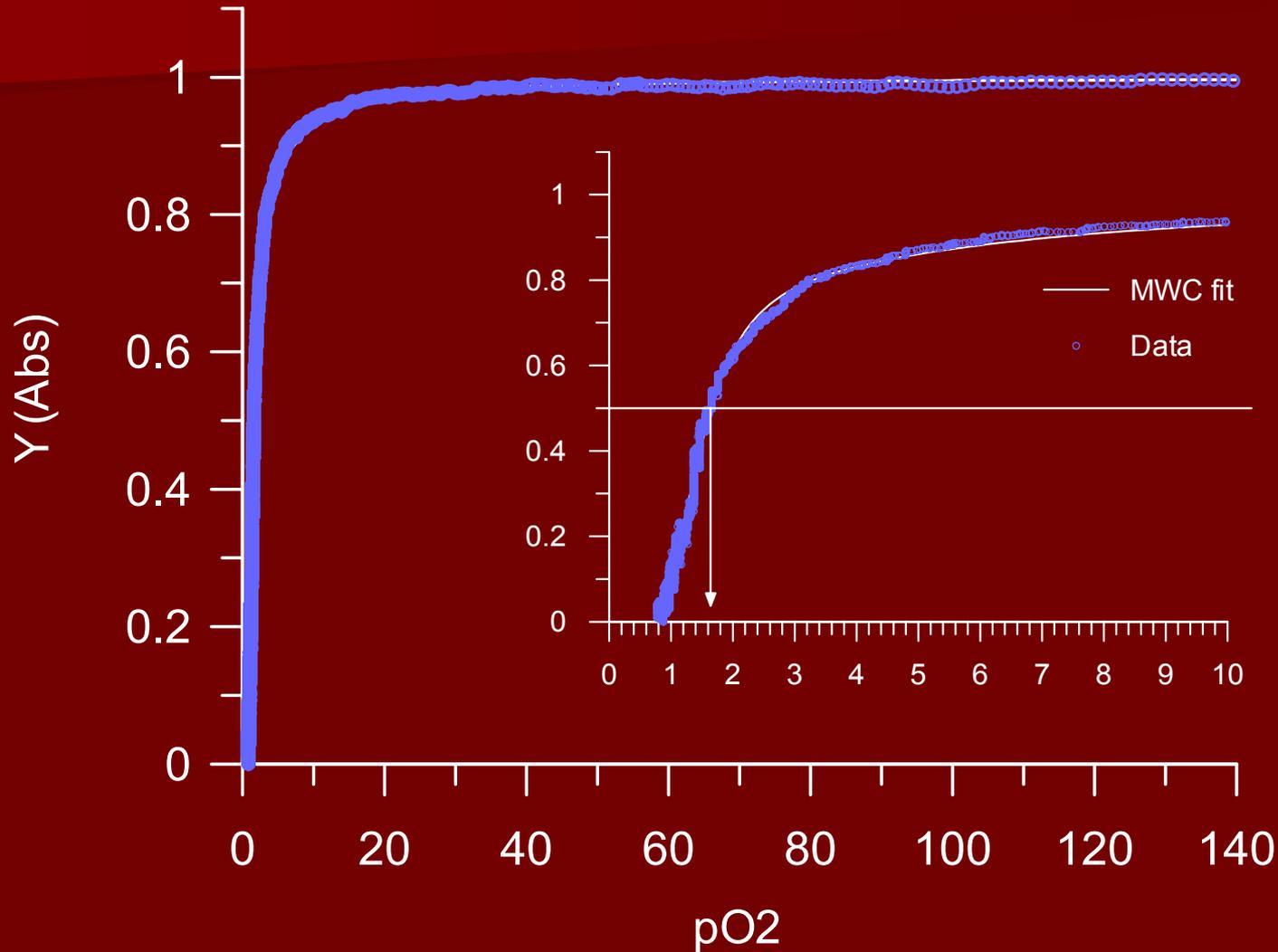
Microsoft Excel - Book1

	A	B	C	D	E
1	Time	pO2	abs		
2	Seconds	V	mV		
3					
4	0	1.4988	-9.6727		
5	1	1.499	-9.8332		
6	2	1.4988	-9.691		
7	3	1.4988	-9.117		
8	4	1.4988	-8.6468		
9	5	1.4998	-8.2926		
10	6	1.5005	-7.9507		
11	7	1.5011	-7.6942		

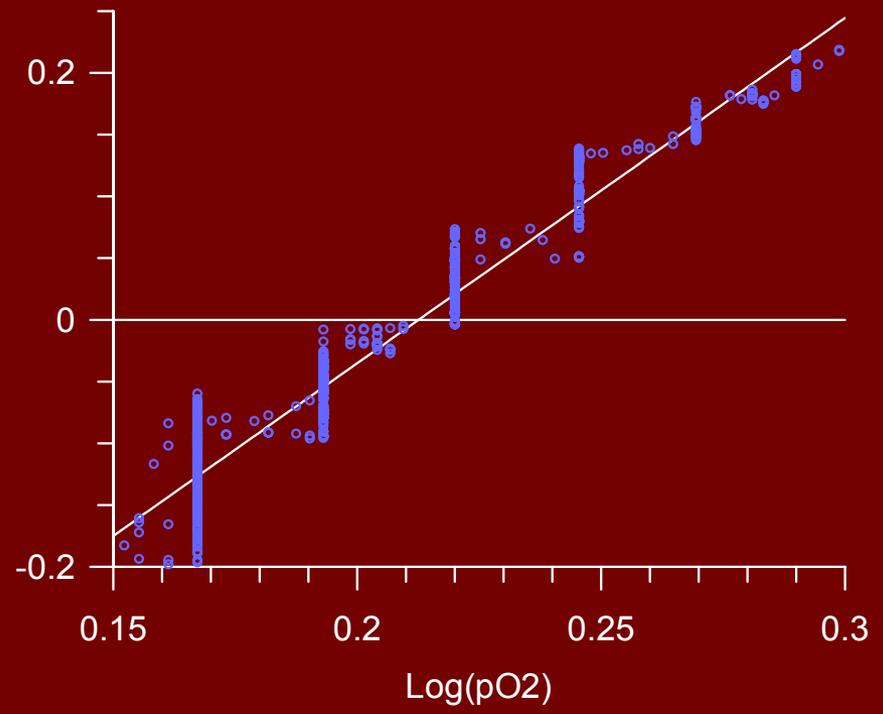
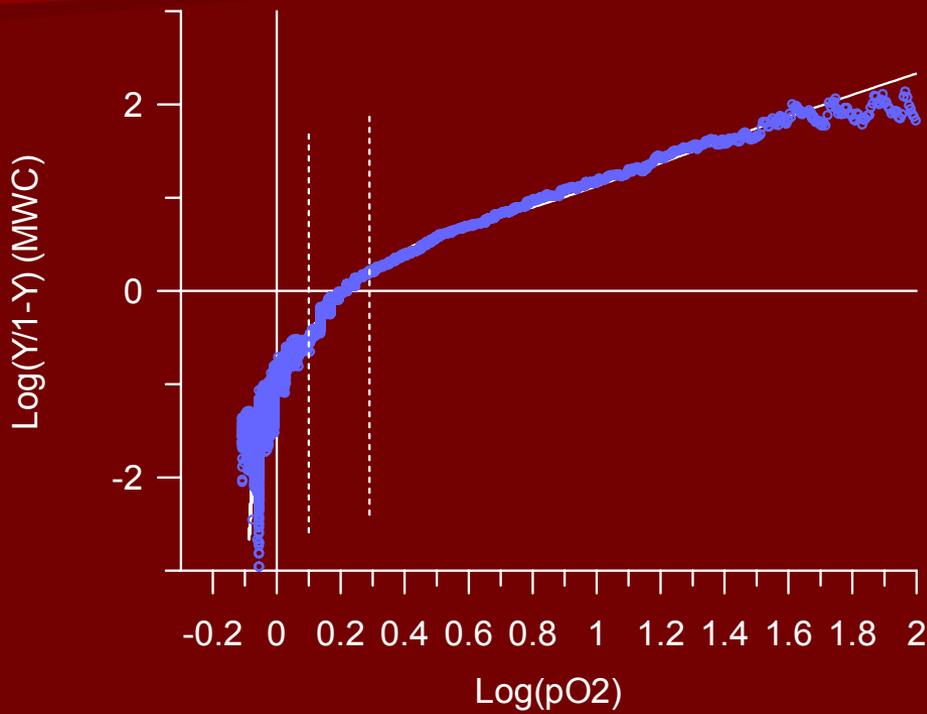
# Data Analysis

- Your pO<sub>2</sub> is recorded in mV so you need to convert into torr units by multiplying it by 100
- Your Abs is recorded as  $1000 * \text{Log}(A^{570}/A^{560})$ , so you will have to convert it into actual absorbance readings by dividing by 1000 and taking anti Log (10 to the power).
- You can do all this calculations in EXCEL

# Sample of native Hb



# Hill Plot



$n_{50} = 2.7956$       error 0.0331  
 $P_{50} = 1.6$