

# *DAAD Scholarship* report

Dr. András Kiss

September 20, 2018

In this document I summarize the most important results of the work I have done in the laboratory of prof. Markus Hoth at the Center of Integrative Physiology and Molecular Medicine (CIPMM) in Homburg during the time period of 11th June 2018 – 10th of August 2018 under the supervision of Dr. Monika Bozem. The work was financially supported by the German Academic Exchange Service (Deutscher Akademischer Austausch Dienst, DAAD).

The main goal of the work was to map the  $H_2O_2$  production of certain immune cells with the Scanning Electrochemical Microscope (SECM). Additionally, new protocols had to be worked out in order to accomplish that goal.

By the time I joined the electrochemistry team at CIPMM, a lot of successful work has already been done that is published in [1]. Upon my arrival, Dr. Bozem presented three recently discovered problems which had to be solved before electroactive species like  $H_2O_2$  and  $O_2$  could be mapped in the close vicinity of the immune cells by the SECM:

- high distortion in the images,
- large amount of noise when the temperature is increased to the physiological 37 °C,
- insufficient resolution of the cells due to the relatively large diameter of the platinum microelectrodes.

We selected the first one to be worked on. The reason for this is that I have experience with reconstructing SECM images by removing distortion based on theoretical models. The problem is shown in Fig. 1B. When a monocyte (Fig. 1A) is scanned with the SECM, the resulting image is distorted. Eventually I solved the problem with deconvolution (Fig. 1C). With this improvement,  $H_2O_2$  and other electroactive species can be mapped with higher temporal resolution. This summary is mostly about how I did it. I managed to improve distorted  $H_2O_2$  images recorded earlier by Dr. Monika Bozem and Phillip Knapp, and conducted my own experiments and applied the deconvolution on those as well. To investigate the problem, first I have reduced the complexity by creating a model system. It featured an „ideal” step, that has a well defined geometry. The system consisted of

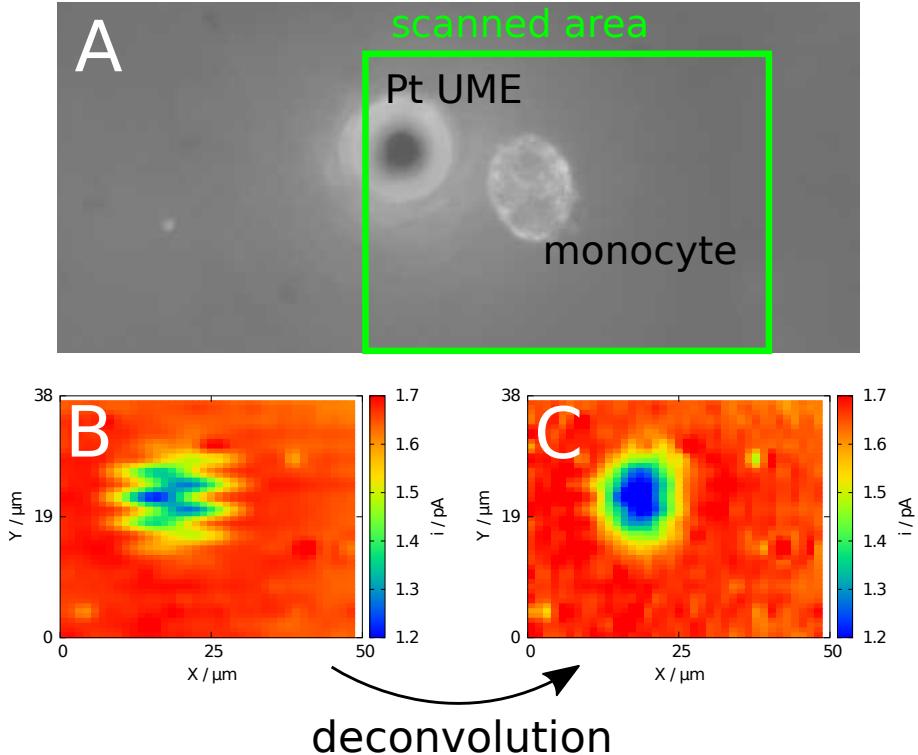


Figure 1: (A) Optical microphoto of the setup including the monocyte, the platinum microelectrode tip and the scanned area. (B) Raw measurement. Values represent the tip current oxidizing  $\text{H}_2\text{O}_2$  at the platinum tip. The distortion is visible along the alternating scanlines. (C) Deconvoluted image obtained from (B).

a broken glass sheet in a Petri-dish. The broken edge of the glass sheet is very sharp, and creates a good step function. When the Pt UME is scanned above it, the measured current is low when the tip is close to the glass sheet, but once it reaches the edge and it is suddenly in the bulk of the solution, the current increases, because diffusion of the electroactive species (ferrocene) is no longer hindered. This is an ideal setup to study how the image of a near perfect step function is distorted by the scanning process. A sketch of the model system can be seen in Fig. 2.

The result of the SECM scanning is presented in Fig. 2B. When the system is scanned with a speed of  $5 \mu\text{m}/\text{s}$ , the image is not distorted. However, when the speed was increased to  $10 \mu\text{m}/\text{s}$ , the image became distorted (Fig. 2C). This speed is still insufficient to investigate a monocyte with fairly good temporal resolution. The distortion is very similar I have encountered during my PhD studies [2, 3]. In that case, the distortion was caused by the relatively large RC time-constant of the potentiometric cell. I could solve that by working out a deconvolution algorithm. In this case, the distortion is caused by slow amperometric response that is associated with the Cottrell-equation, but also with the RC time-constant. Therefore the transfer function describing the distortion includes at least two factors:  $1/\sqrt{t}$  from the Cottrell-equation and  $e^{-t/RC}$  from the time constant of the

RC circuit element that is present in the amperometric cells as well as potentiometric cells. The deconvolution function is the inverse of those functions. I have written the following Python program to perform the deconvolution:

```

1 #!/usr/bin/enc python
2
3 # Here is a first attempt at porting the deconvolution algorithm
4 # from FORTRAN to python and applying it on amperometric SECM images.
5 # The gaussian filter is not yet implemented in the program.
6 # Right now I do it with the plotting software (gnuplot),
7 # but it would be better if the python program did it. Also, I haven't
8 # done the command line argument interpreter yet, so the file name must
9 # be changed in the code every time. A GUI would be nice, and a live plot
10 # of the convoluted and deconvoluted image. For that, the XYZ data needs
11 # to be converted to a matrix.
12
13 import numpy as np
14
15 conv_img = np.loadtxt("11.txt")
16 deconv_img = np.copy(conv_img)
17 e0 = np.float32(conv_img[0][2])
18 for n in range(0, conv_img.shape[0]):
19     deconv_img[n][2] = np.float32((conv_img[n][2] - e0 * 0.985) / (1 - 0.985))
20     e0 = np.float32(conv_img[n][2])
21
22 np.savetxt("11_python_deconvoluted.txt", deconv_img, delimiter=" ")

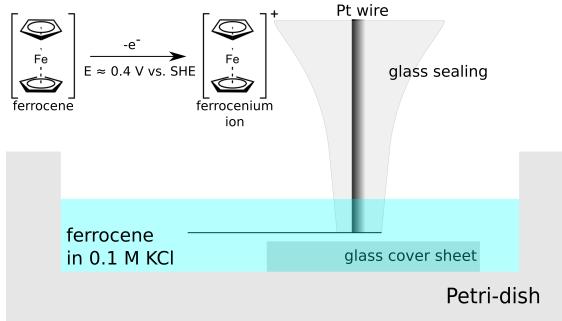
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Quite surprisingly, after deconvolution, the image became even more distorted (Fig. 2D). The explanation is the following. It is a well known fact from hydrodynamics, that when two planes close to each other move relatively to each other in a liquid, and when one reaches the edge of the other, a very strong convective effect is present. This is depicted in Fig. 2E. This effect increases the current for a short time when the electrode is moving from above the glass sheet cover to the bulk. The change is more sudden than when it is moving in the opposite direction and it negates the delaying effect.

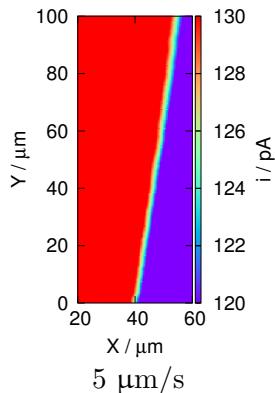
I had to find another model system that acts as a step function. I have built a model that is depicted in Fig. 3A. I fixed a  $d = 10 \mu\text{m}$  Pt wire on the bottom of a Petri-dish. I filled the dish with ferrocenium solution with KCl as background electrolyte. The microphoto of the completed system taken from below can be seen in Fig. 3B. Then I approached the wire with the ultramicro-electrode and scanned perpendicularly. Of course this is not an ideal step function, but actually models a cell better. The resulting image is plotted in Fig. 3C.

The deconvolution on this system worked. The expected shape – that is a straight stripe across the image – is restored (Fig. 3D). The distortion caused by the long response time of the cell is reduced. The deconvolution function is certainly far from perfect, and

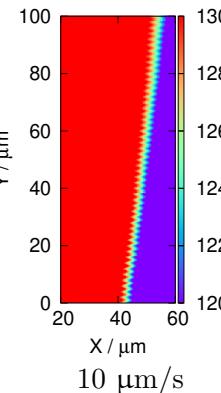
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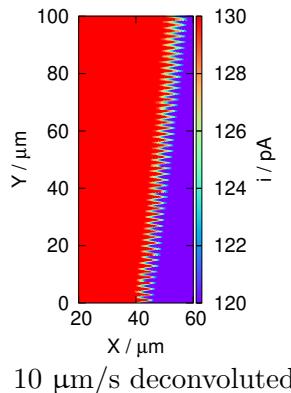
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D



E

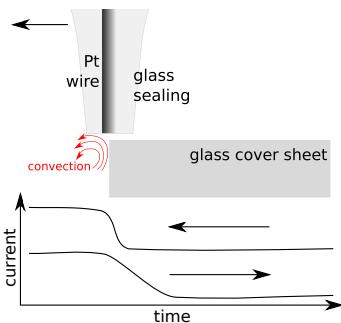
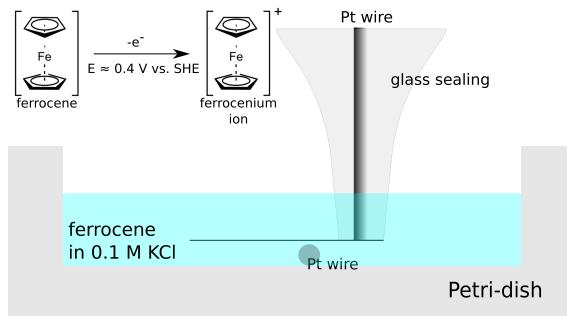
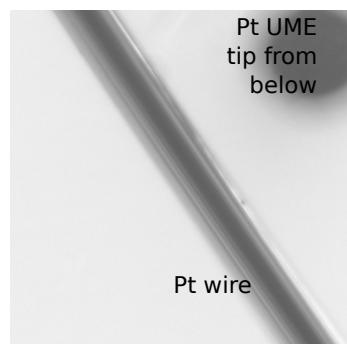


Figure 2: The model system to study a simple step function. The oxydation current through the tip is low when the electrode is above the class sheet because of hindered diffusion. The current increases as the tip moves towards the bulk. The transition should be a sharp step function. (A) Sketch of the system. (B) SECM scan result at 5  $\mu\text{m}/\text{s}$ . (C) SECM scan result at 10  $\mu\text{m}/\text{s}$ . As it can be seen, the image is a lot more distorted at 10  $\mu\text{m}/\text{s}$  than at 5  $\mu\text{m}/\text{s}$ . (D) Attempted deconvolution. The deconvolution in this case did not work, because the raw image was heavily influenced by convection (E). Explanation is in the text.

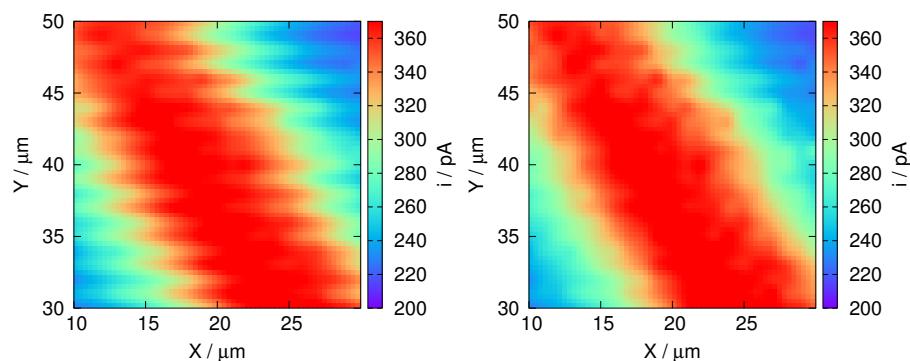
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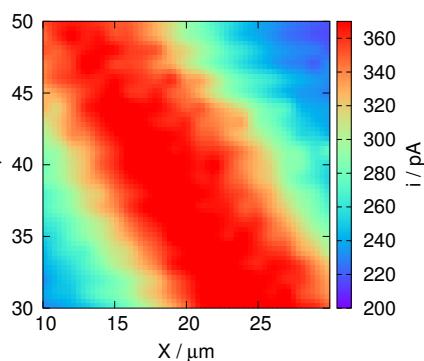


Figure 3: (A) Sketch of the second model system. In this system I placed a  $d = 10 \mu\text{m}$  Pt wire on the bottom of a Petri-dish and I scanned above it with a Pt microelectrode. Electrolyte was KCl, electroactive species was ferrocenium. (B) Optical microphoto of the system. (C) Raw image. (D) Deconvoluted image.

the distortion is a lot more complex. The image however improved, and I consider this result a good starting point.

In the remainder of the report I show the results of the deconvolution of earlier SECM images of immune cells recorded by Dr. Monika Bozem and Phillip Knapp as well as the deconvolution of measurements I have done. This is a big advantage of deconvolution compared to other techniques that aim to reduce distortion: deconvolution can be applied *ex post facto*. I have already shown an example of mapping H<sub>2</sub>O<sub>2</sub> above a monocyte in Fig. 1B. The scanning tip was a 10 μm Pt microelectrode. The optical microphoto of the scanned monocyte can be seen in Fig. 1A. The resulting image is distorted (Fig. 1B). After I ran the program above on the dataset plotted in Fig. 1B, the distortion decreased significantly (Fig. 1C). I have managed to successfully deconvolute distorted amperometric SECM images of human immune cells recorded with the HEKA ElProScan SECM system.

The SECM image shown in Fig. 1B was measured with an electrode manufactured by Phillip Knapp, PhD student supervised by Dr. Monika Bozem. I have also constructed my own Pt microelectrodes for further experiments. The so-called *RG*-value is a very important parameter of the amperometric microelectrodes. This is the ratio of the diameter of the whole electrode at the tip (insulation + electroactive part) and the electroactive part. I have managed to create Pt UMEs (ultramicro electrodes) with an RG-value of ≈ 2.5. I have used the manufactured electrodes in an interesting application that I have introduced in prof. Hoth's laboratory. Previously H<sub>2</sub>O<sub>2</sub> was measured, because this can be easily done, and it is a good indicator of the general state of a cell undergoing oxidative stress. It is also considered a signalling molecule in the immune system. I've asked Dr. Bozem if it would be interesting to know the concentration of oxygen in the close vicinity of cell. She told me it would be very interesting, so I designed an experiment to demonstarte it. I have mapped the oxygen partial pressure of a human monocyte with the SECM. This can provide information about their metabolic activity. It has great importance in the immune system, where an oxidative burst can significantly influence the O<sub>2</sub> concentration nearby the cell. In all cases, oxygen partial pressure is decreasing in the close vicinity of the cell either as a result of cell breathing or increased oxygen uptake to make the oxidative burst possible (Fig. 4A).

I scanned a human monocyte (optical microphoto in Fig. 4B) at about 3 μm height above the topmost point of the cell. The tip potential was set to -700 mV vs. the Ag/AgCl quasi-reference electrode. At this potential the platinum microelectrode reduces oxygen. The result of the oxygen mapping can be seen in Fig. 4C. Once again, the image is distorted. After deconvolution however, the expected image is acquired: a circular shape with a much less distorted contour (Fig. 4D). The scanning distortion is almost completely removed in the low concentration region (red), and significantly reduced in the high oxygen concentration region (green-blue). I have calculated the corresponding oxygen partial pressure values based on the linear relationship between the tip current and the oxygen partial

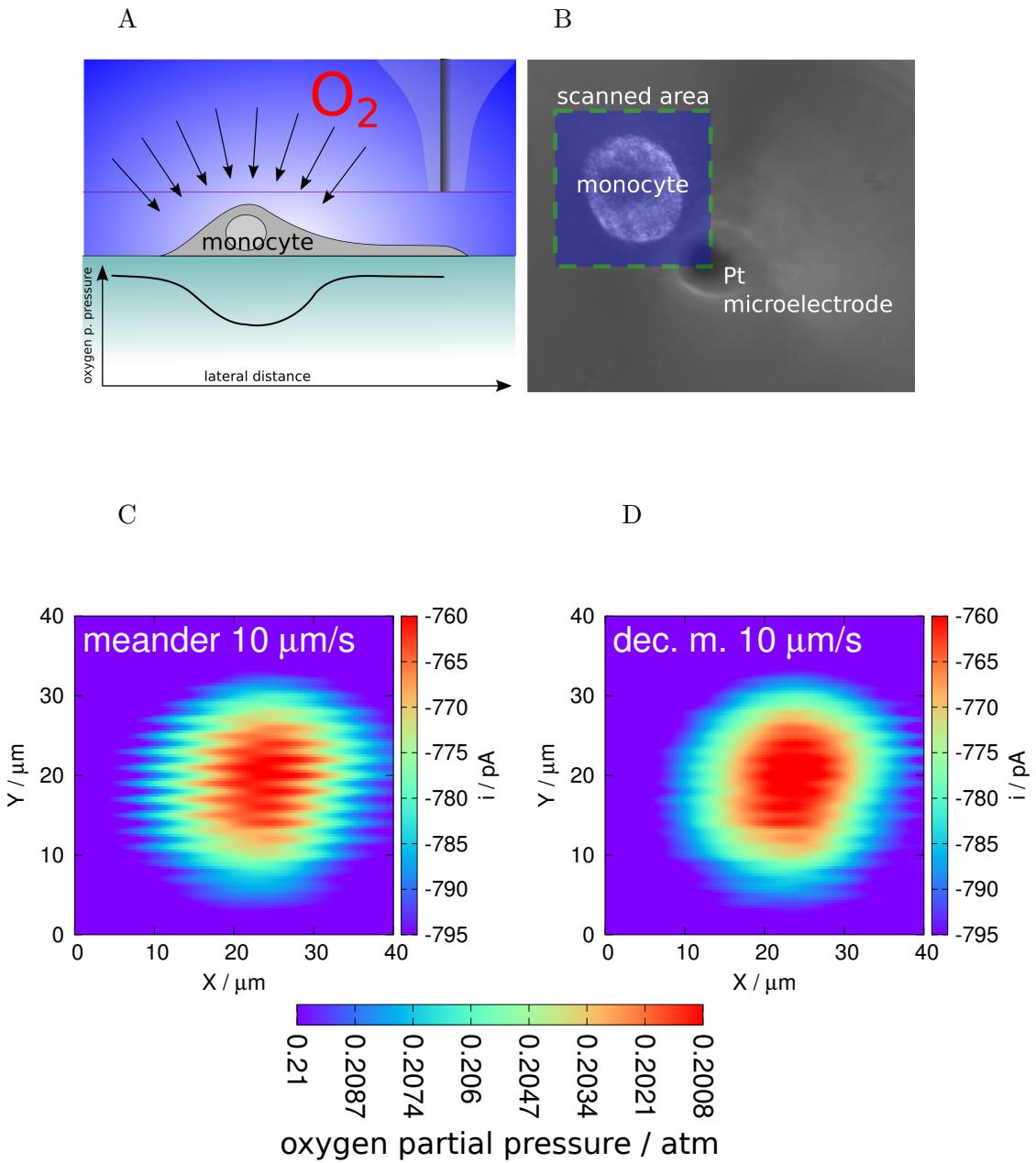


Figure 4: (A) Sketch of the oxygen mapping above the human monocyte. (B) Optical microphoto indicating the monocyte, the scanning tip ( $10 \mu\text{m}$  Pt) and the scanned area. (C) Raw scan. (D) Deconvoluted image.

pressure. As it can be seen, the decrease is very small, but still very much detectable with this very powerful electroanalytical method.

To summarize this report, I have managed to map hydrogen-peroxyde and oxygen concentration above human immune cells with the SECM. These are two very important species in the immune system, as one is an important signal molecule, and both of them are participating in an oxidative burst. Furthermore, oxygen consumption is a very good indicator of the current metabolic state of a cell. To show that the deconvolution works, I had to reduce the geometry. I have constructed two model systems, and shown that the deconvolution indeed works.

These are my most important results, and I have included only these as was requested. However, I have done a lot more about several different small topics. Therefore I am attaching below the scanned version of the notebook I have written about my work.

Finally I would like to thank DAAD for making my stay at the University of Saarland possible by providing financial support. The support is greatly appreciated.

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## References

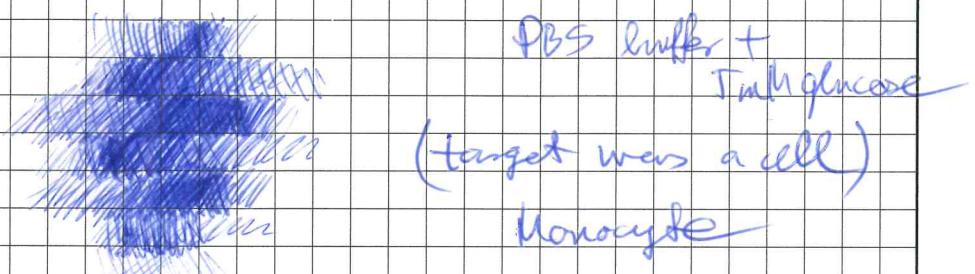
- [1] Monika Bozem, Phillip Knapp, Valentin Mirčeski, Ewa J Slowik, Ivan Bogeski, Reinhard Kappl, Christian Heinemann, and Markus Hoth. Electrochemical quantification of extracellular local h<sub>2</sub>o<sub>2</sub> kinetics originating from single cells. *Antioxidants & redox signaling*, 29(6):501–517, 2018.
- [2] András Kiss and Géza Nagy. Deconvolution in potentiometric secm. *Electroanalysis*, 27(3):587–590, 2015.
- [3] András Kiss and Géza Nagy. Deconvolution of potentiometric secm images recorded with high scan rate. *Electrochimica Acta*, 163:303–309, 2015.

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This is an attempt to deconvolute an alot raster scan measured by Dr. Monika Boren and Phillip Knapp, on 2014.01.28. This is an ansys-metric raster scan, showing meander distortion.



$E = 670 \text{ mV}$  vs.  $\text{Ag}/\text{AgCl}$   
measuring  $\text{H}_2\text{O}_2$

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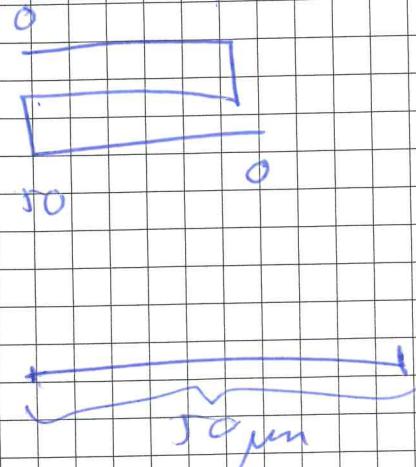
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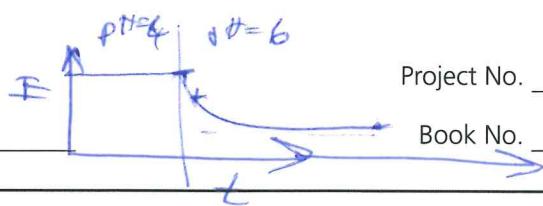
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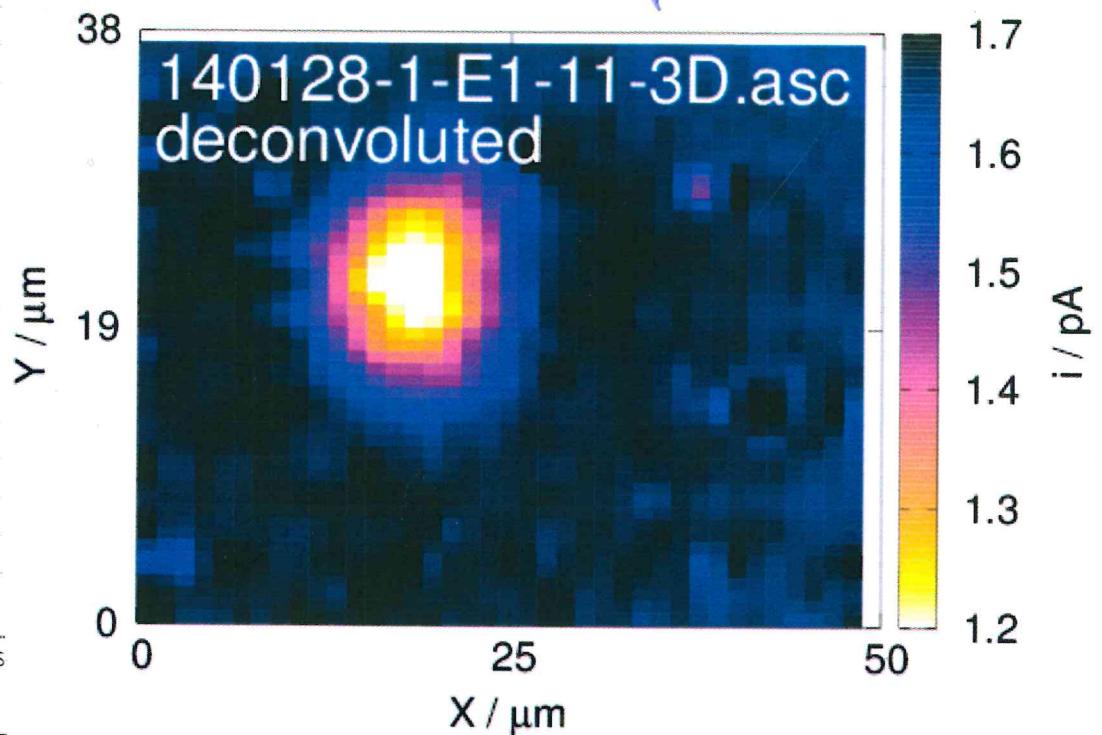
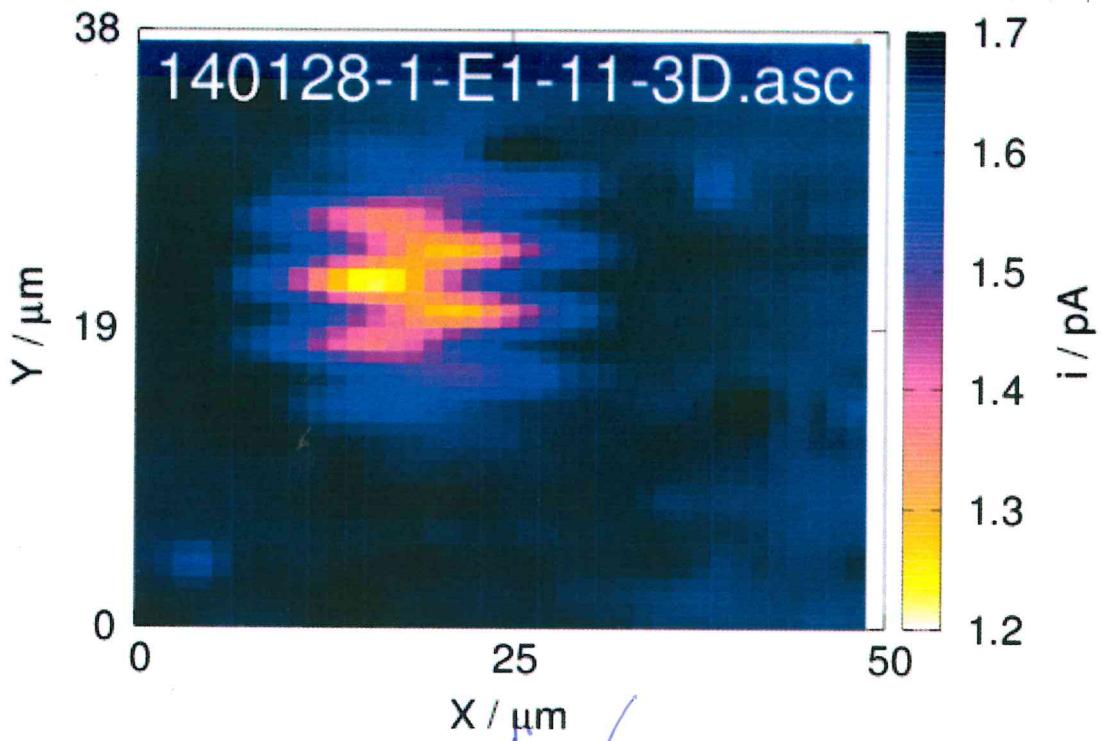
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$$E_t = (E_0 - E_{\infty}) e^{-\frac{t}{Rc}} + E_{\infty}$$

$$E_t = E_0 + [E_0 - E_{\infty}] \cdot e^{-\frac{t}{Rc}}$$



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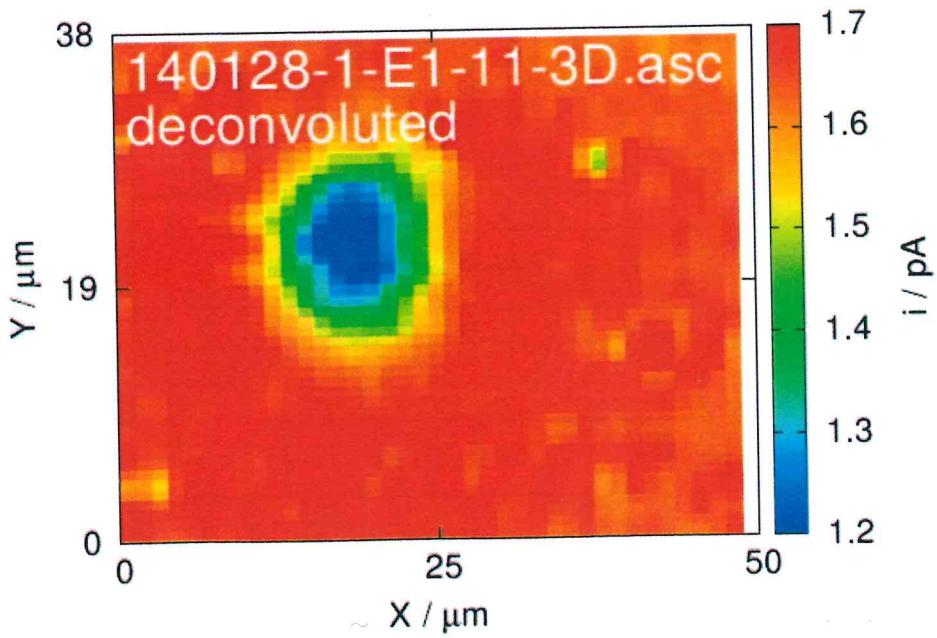
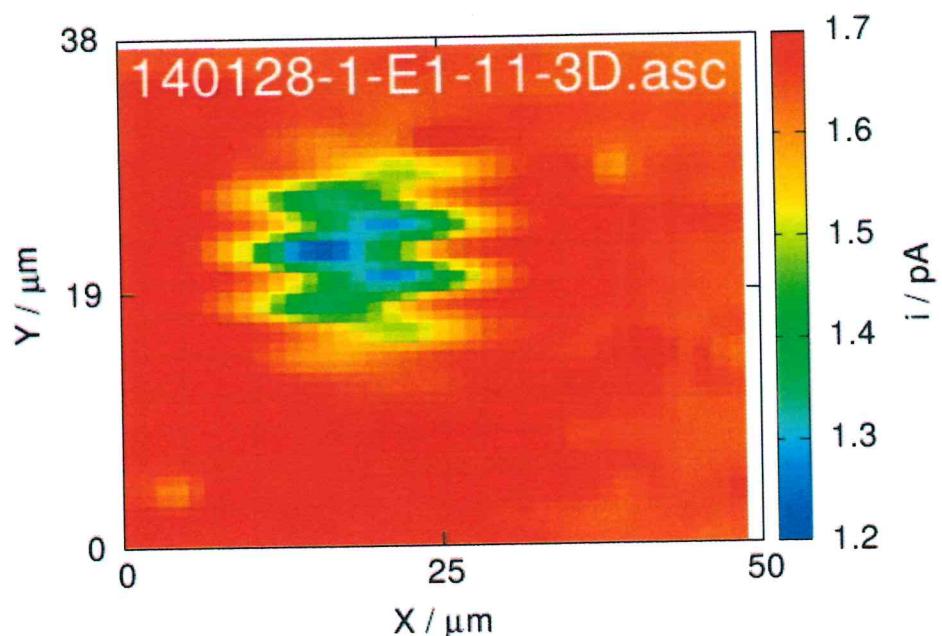
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1) Nernst-equation

$$P_p = \frac{RT}{4\pi} \lg \left( \frac{P_{O_2}}{P_{O_2}^0} \right) + \text{const.}$$



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## Labseminar 2018

Monday at 11.00

(Biophysics)

Auditorium CIPMM

*Presentation*

January, 8 <sup>th</sup>	-----	August, 6 <sup>th</sup>	Dalia
January, 15 <sup>th</sup>	Markus	August, 13 <sup>th</sup>	Girish
January, 22 <sup>nd</sup>	Leticia	August, 20 <sup>st</sup>	Diana
January, 29 <sup>th</sup>	Katerina	August, 27 <sup>th</sup>	Reinhard
February, 5 <sup>th</sup>	Bin	September, 3 <sup>rd</sup>	Janina
February, 12 <sup>th</sup>	<b>no seminar</b>	September, 10 <sup>th</sup>	Lea
February, 19 <sup>th</sup>	Kim	September, 17 <sup>th</sup>	Anni
February, 26 <sup>th</sup>	Arne	September, 24 <sup>th</sup>	Maylin
March, 5 <sup>th</sup>	Renping	October, 1 <sup>st</sup>	Lucas
March, 12 <sup>th</sup>	Eva	October, 8 <sup>th</sup>	Carsten
March, 19 <sup>th</sup>	Mona	October, 15 <sup>th</sup>	Nikolina
March, 26 <sup>th</sup>	<b>no seminar</b>	October, 22 <sup>nd</sup>	Monika
April, 9 <sup>th</sup>	Monika	October, 29 <sup>th</sup>	Michelle
April, 16 <sup>th</sup>	general points	November, 5 <sup>th</sup>	Phillip
April, 23 <sup>rd</sup>	Maik	November, 12 <sup>th</sup>	Adrian
April, 30 <sup>th</sup>	<b>no seminar</b>	November, 19 <sup>th</sup>	Julia
May, 7 <sup>th</sup>		November, 26 <sup>th</sup>	Sylvia
May, 14 <sup>th</sup>	Jie Zhu	December, 3 <sup>rd</sup>	Barbara N.
May, 28 <sup>th</sup>	Ewa J.	December, 10 <sup>th</sup>	
June, 4 <sup>th</sup>	Denise	December, 17 <sup>th</sup>	
June, 11 <sup>th</sup>	Remy		
June, 18 <sup>th</sup>	Vanessa		
<b>June, 25.-Aug, 3.</b>	<b>no seminar</b>		

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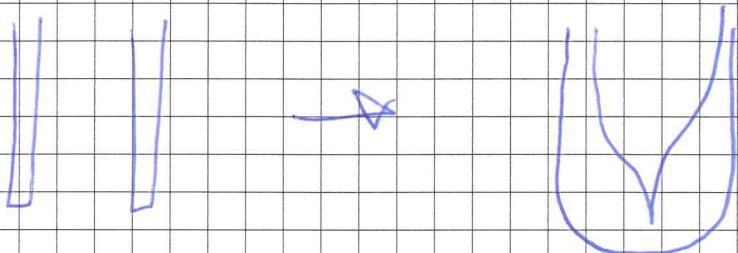
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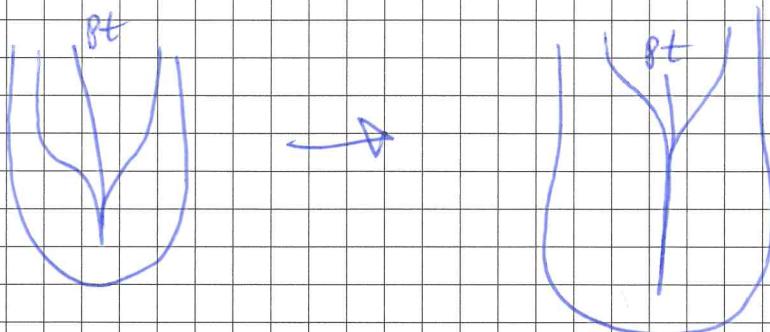
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I wanted to show Marlon and Phillip how do we prepare the UMEs in  $\mu\text{m}$ .

First, we sealed a  $d_o=2\text{mm}$   $d_i=1\text{mm}$  borosilicate capillary at one end:



Then, I put in the  $R \approx 1\text{cm}$   $d = 10\mu\text{m}$  Pt wire, and seal it with a propane-butane burner:



Then, I push the solder into the capillary close to the Pt wire. After that I melted it in the same flame

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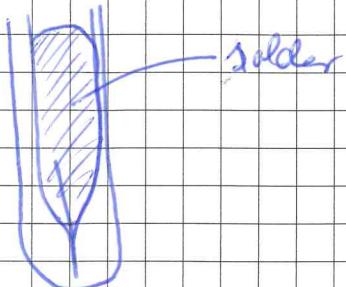
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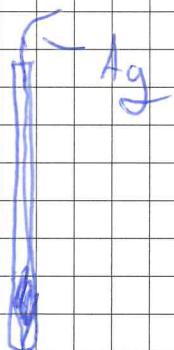
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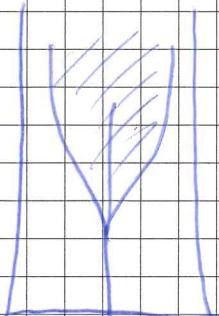
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Then, while the solder was still molten,  
I pushed in an 0.8 cm silver wire  
to provide electric connection to the proton-  
tintat



Then, I ground the sealed end to  
expose the Pt-wire.



To Page No. \_\_\_\_\_

Witnessed and understood by me

Date

Invented by

Date

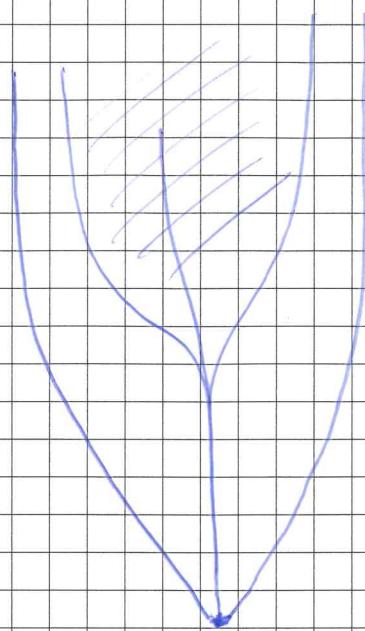
Recorded by

TITLE \_\_\_\_\_

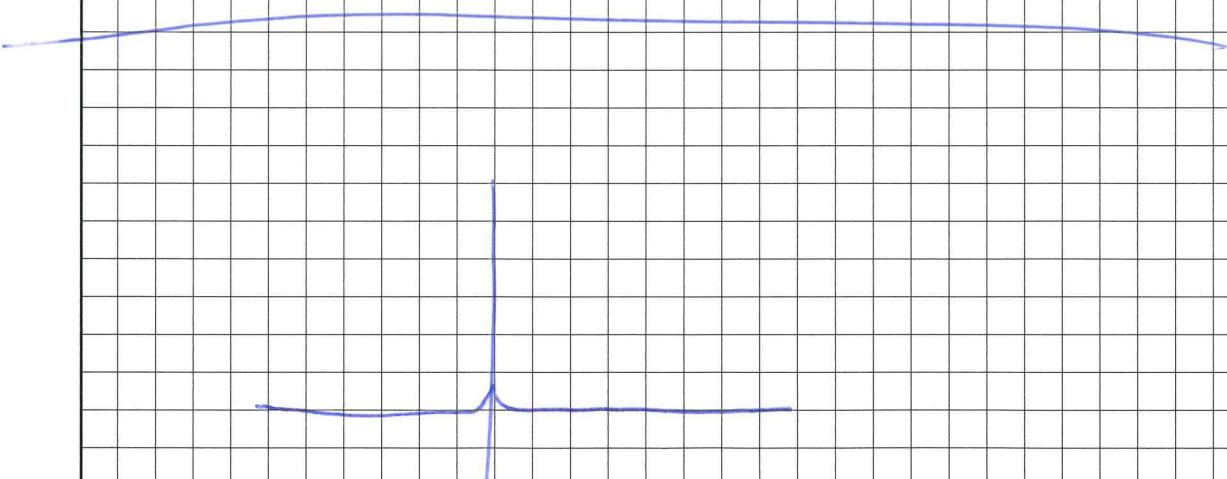
Book No. \_\_\_\_\_

From Page No. \_\_\_\_\_

Then, I ground the benzene:



Tested with CV in 2mM ferrocene / 100mL KCl.



To Page No. \_\_\_\_\_

Witnessed and understood by me

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Invented by

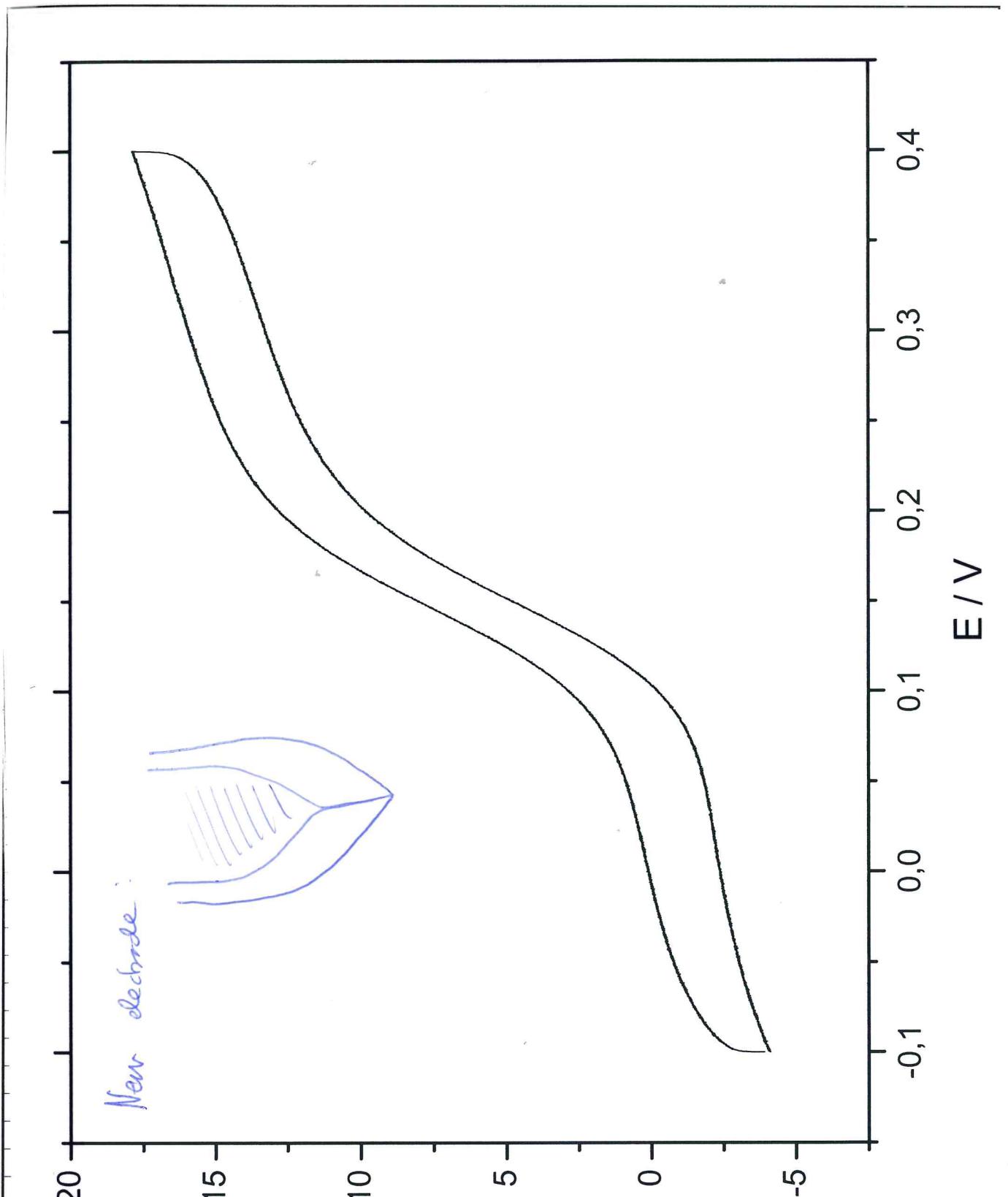
Date

Recorded by

Project No. \_\_\_\_\_

TITLE \_\_\_\_\_

Book No. \_\_\_\_\_



10 Page No. \_\_\_\_\_

Witnessed and understood by me

Date

Invented by

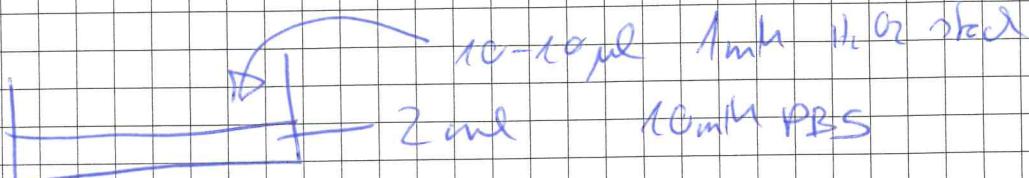
Date

Recorded by

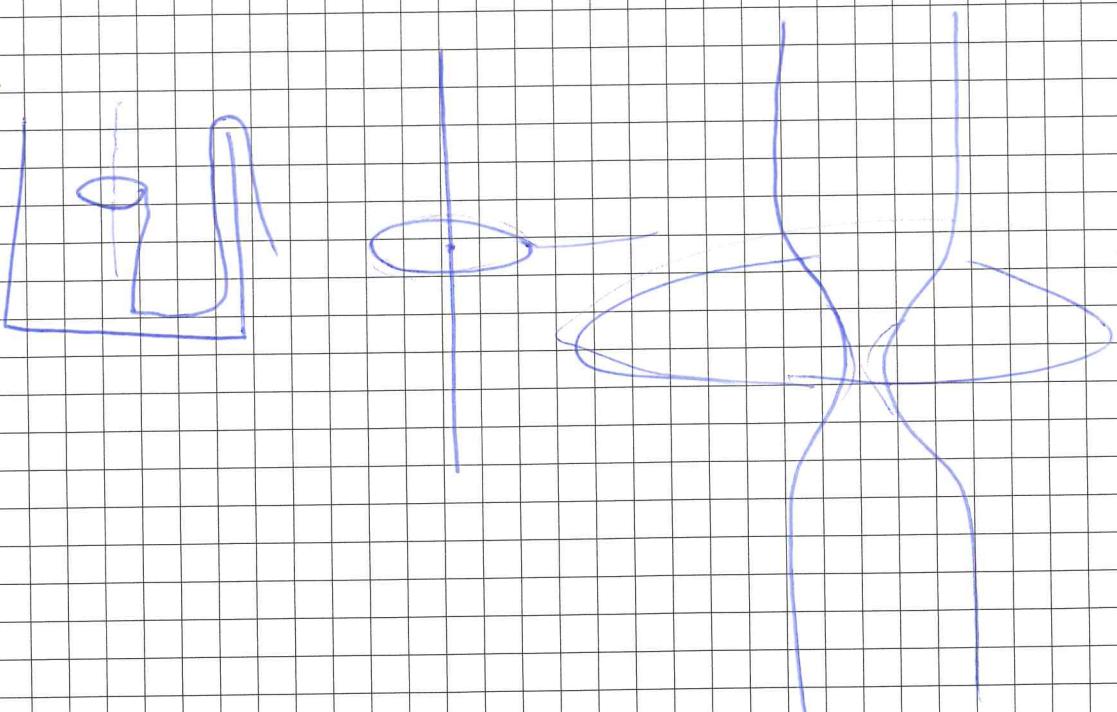
TITLE  $H_2O_2$  chronoamperometric calibration

From Page No. \_\_\_\_\_

With the new electrode.



10-10 $\mu$ l 1mM  $H_2O_2$  stock  
1st  
2nd 10mM PBS

increase in  $H_2O_2$  : 5  $\mu$ l each addition24.5°C $H_2O_2$  stock solution prepared by Phillips

To Page No. \_\_\_\_\_

Witnessed and understood by me

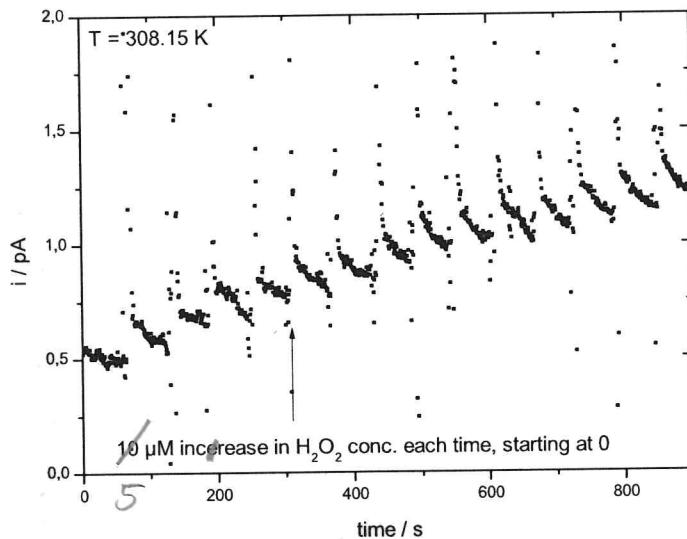
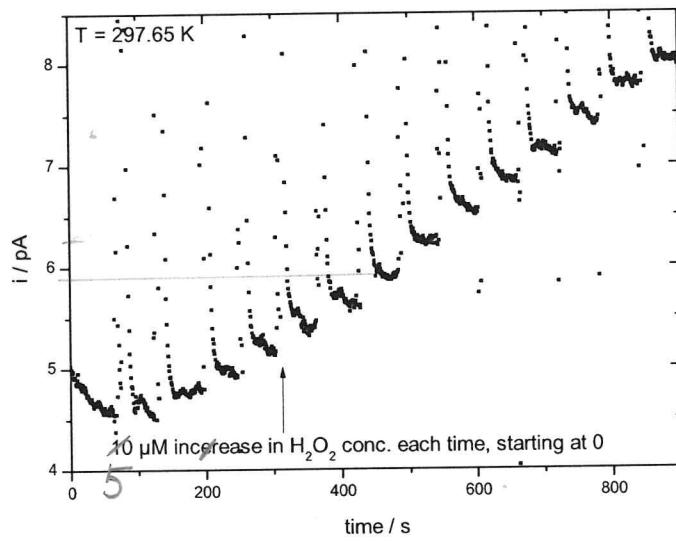
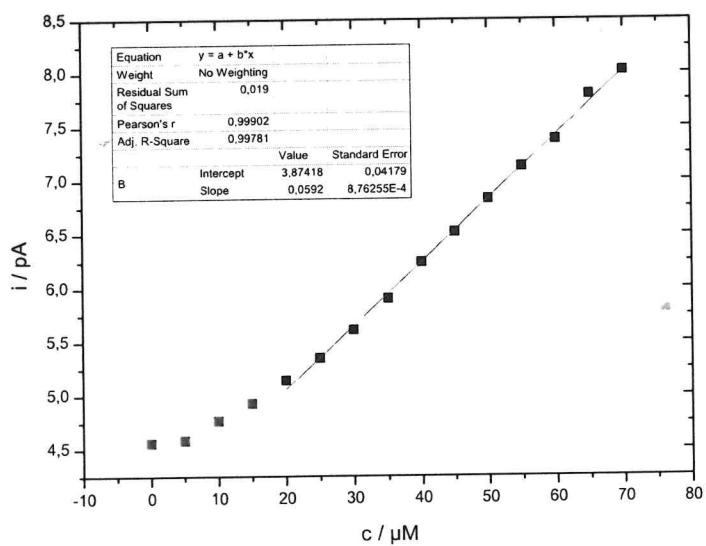
Date

Invented by

Date

Recorded by

From Page No. \_\_\_\_\_



To Page No. \_\_\_\_\_

Witnessed and understood by me

Date \_\_\_\_\_

Invented by \_\_\_\_\_

Date \_\_\_\_\_

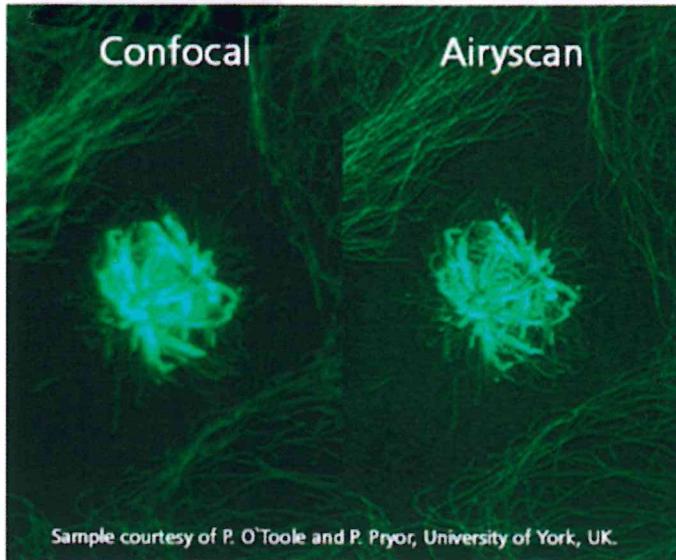
Recorded by \_\_\_\_\_

Project No. \_\_\_\_\_

TITLE Zein Wahby

Book No. \_\_\_\_\_

From Page No. \_\_\_\_\_



Sample courtesy of P. O'Toole and P. Pryor, University of York, UK.

ZEISS Airyscan is revolutionizing confocal imaging.



Download the free white paper to learn how this new detector concept for confocal provides higher signal-to-noise, less bleaching, faster imaging and super resolution with any fluorophore.

140514-1.dat

① 6

E1-1, maybe

beads

E1-3

To Page No. \_\_\_\_\_

Witnessed and understood by me

Date

Invented by

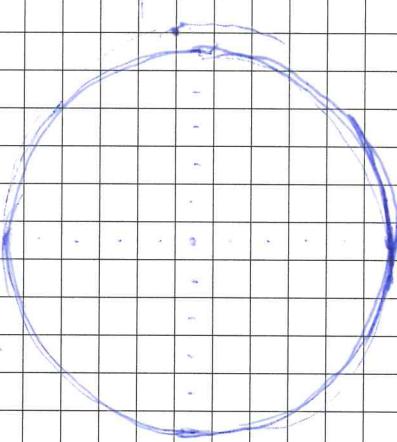
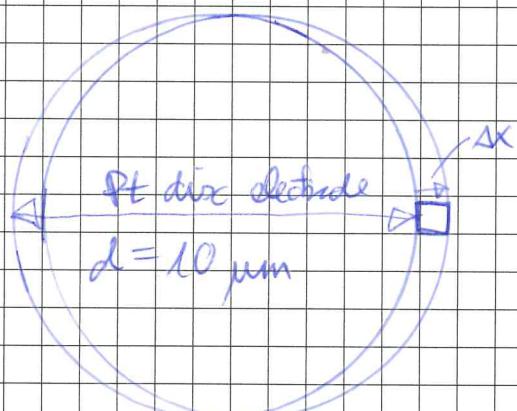
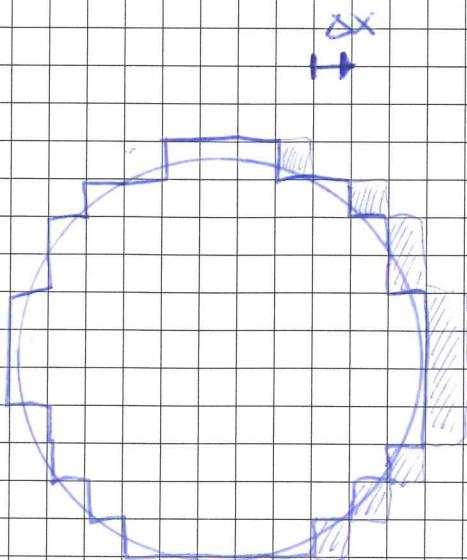
Date

Recorded by

TITLE \_\_\_\_\_

Book No. \_\_\_\_\_

From Page No. \_\_\_\_\_



To Page No. \_\_\_\_\_

Witnessed and understood by me

Date

Invented by

Date

Recorded by

TITLE 140514-1.dat

Book No. \_\_\_\_\_

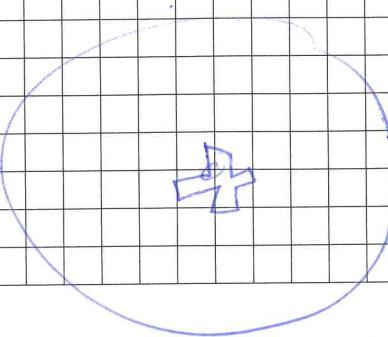
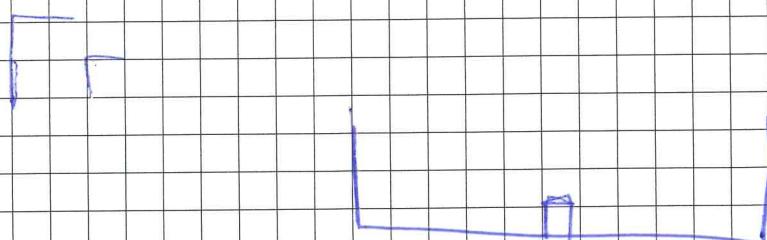
From Page No. \_\_\_\_\_

E1-745 μm X 65 μm2 μm/sμ step : 2 μm26 scanlinesX: 0 - 45 (401 row)y:lines in the file: 13026

$$\frac{13026}{466} = 26$$

13026  
466  
26

TO

401 45 μm X 85 μm


To Page No. \_\_\_\_\_

Witnessed and understood by me

Date

Invented by

Date

Recorded by

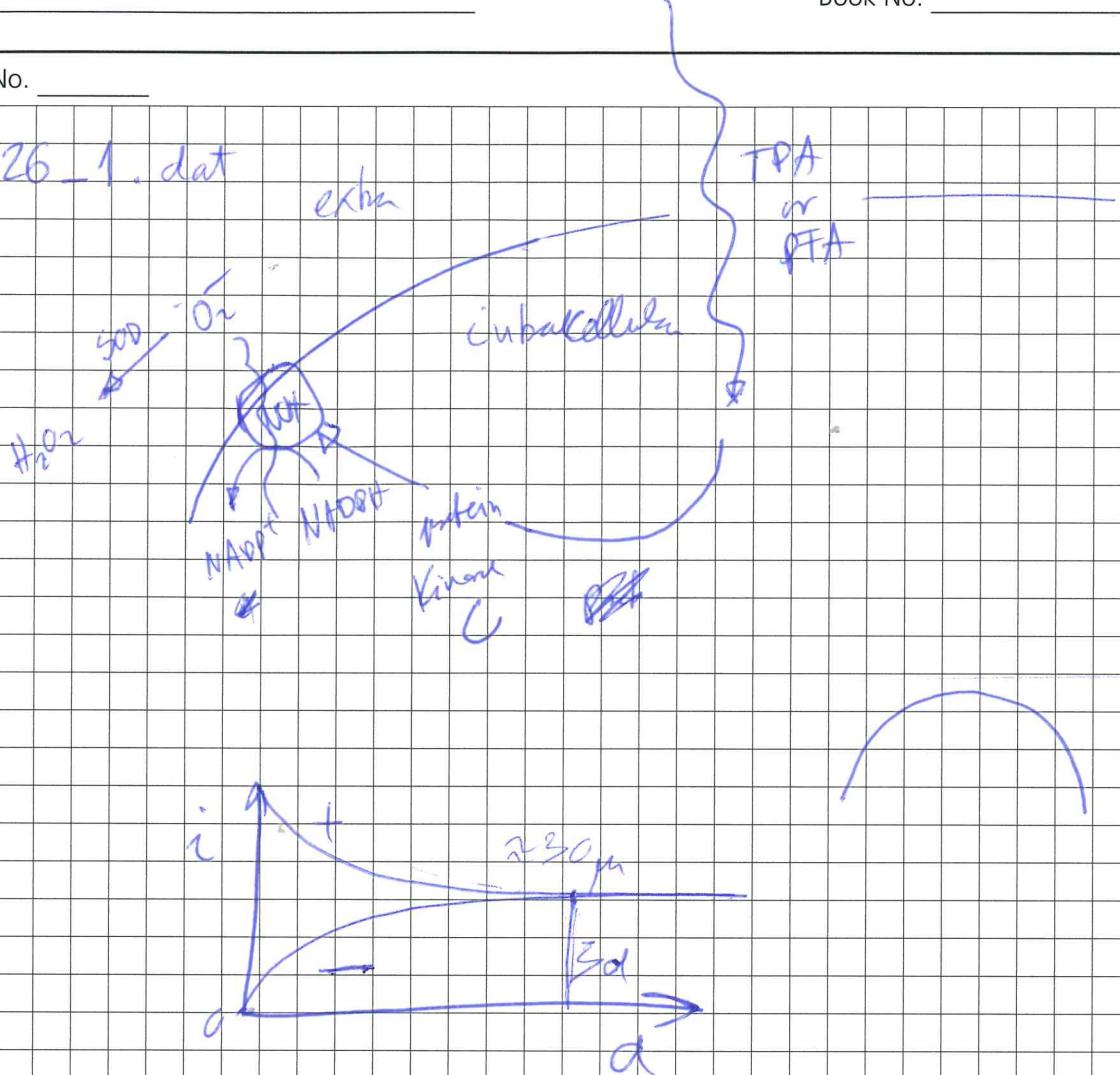
TITLE \_\_\_\_\_

Book No. \_\_\_\_\_

From Page No. \_\_\_\_\_

M1

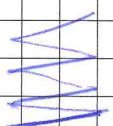
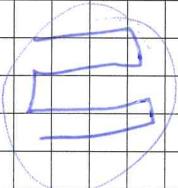
18626-1.dat



cell attached to UTP and dragged along

E1-11-4

0.5 mM TPA  
+ 5 μl to 2 ml



1 μM TPA for the cells  
in DMSO

To Page No. \_\_\_\_\_

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Date

Invented by

Date

Recorded by

From Page No. \_\_\_\_\_

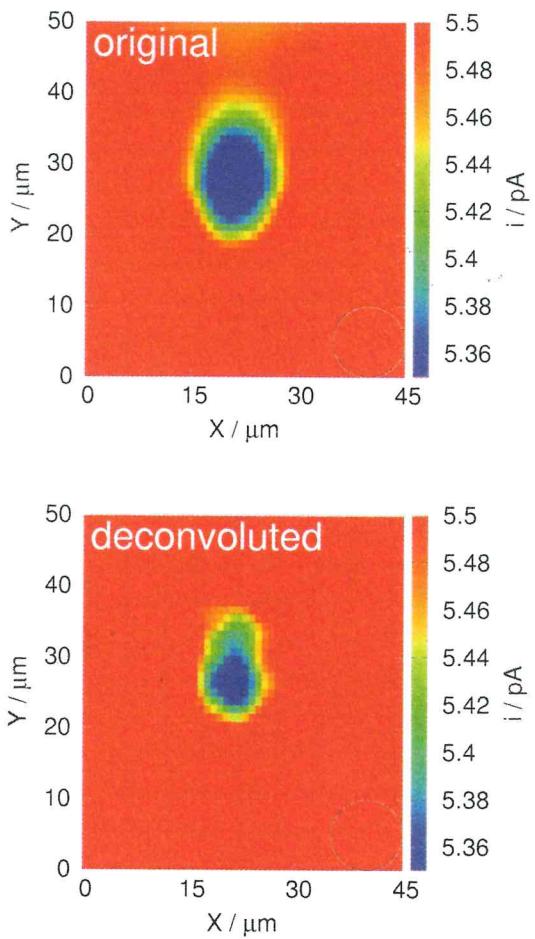


Figure 1

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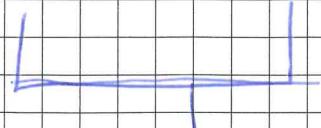
Date

Initial  
Recorded

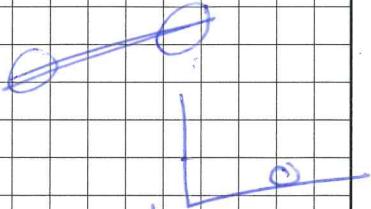
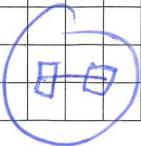
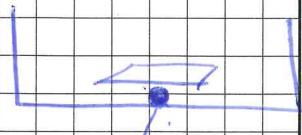
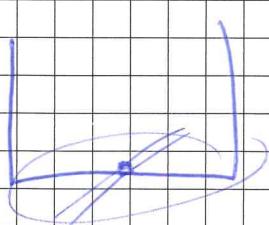
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www.wpiinc.com

TITLE 180627 New cell experiment

From Page No. \_\_\_\_\_

1, Wash cell culture 2ml PBS

fibronectin  
+  
cells

Put 2ml PBS in2, Place electrodes3, Load for cells (jacketed mode)4, Set origin5, clean electrode 970 pA mV (-70 pA)for Pan records1 μM TPARun

To Page No. \_\_\_\_\_

Witnessed and understood by me

Date

Invented by

Date

Recorded by

*target*  
 TITLE Pt wire measurements for  
spectral deconvolution

From Page No. \_\_\_\_\_

3: meander  
 4: fast comb

10  $\mu\text{m/s}$  200 mV 5 mV/ $\mu\text{A}$

10  $\mu\text{m} \times 50 \mu\text{m}$

1  $\mu\text{m} \times 1 \mu\text{m}$ , stepwise

51  $\times$  51  $\mu\text{m}^2$  0 - 50

5:



5  $\mu\text{m/s}$

meander

6:

best not for  
2 electrode

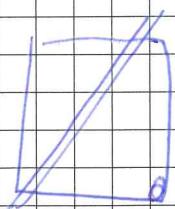
5  $\mu\text{m/s}$

51  $\times$  51

7:

even better

$\Delta t = 5 \mu\text{m}$



start photo: 007

8:

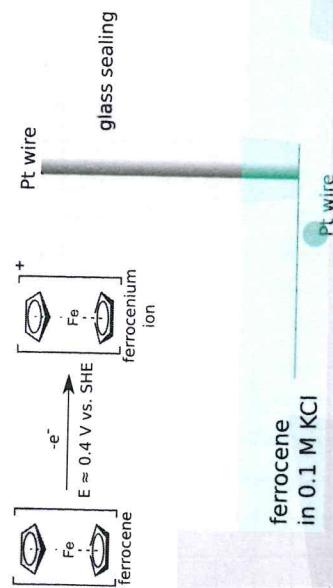


$\phi$  20  $\mu\text{m}$  Pt

start: 010

electrode focus: 5.85  $\mu\text{m}$   
 wire edge focus: -3.82  $\mu\text{m}$

stop: 011



To Page No. \_\_\_\_\_

Witnessed and understood by me

Date

Invented by

Date

180627

Recorded by

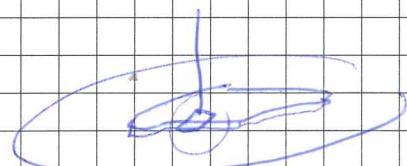
TITLE Image of broken microscope slide cover Book No. \_\_\_\_\_

From Page No. \_\_\_\_\_

ZnM ferrocene in 0.1M KCl



E1-2 - 50x70  
μm μm  
- 1 μm x 1 μm



E1-3 —  
fast comb

E1-4 —  
fast comb 5 μm/s start end picture: 004

E1-5 —  
meander 50 μm/s

E1-6 —  
meander 100 μm/s

~~m. f.c.~~  
2.1 15 ✓ 16 ✓  
2 3 ✓ 4 17 ✓  
10 2 19 ✓ 3 18 ✓  
20 5 16 ✓  
100 6

E1-13: finish: 076

E1-17: finish: 8

E1-32: finish 13

5 μm/s   
10 μm x 10 μm

1 μm x 1 μm res.

E1-33: finish 14

~~40x101~~ To Page No. \_\_\_\_\_~~201~~ 1x1 Date

Witnessed and understood by me

Date

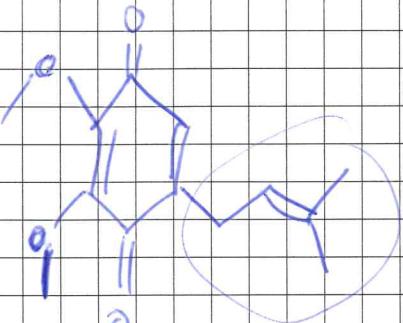
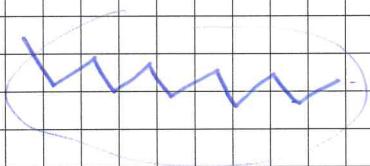
Invented by

180628 Recorded by

From Page No. \_\_\_\_\_

$\text{Ca}^{2+}$  affinity of decylubiquinone in organic solvents

(Valentin Mirceski (SMM)  
Lovic was his supervisor)

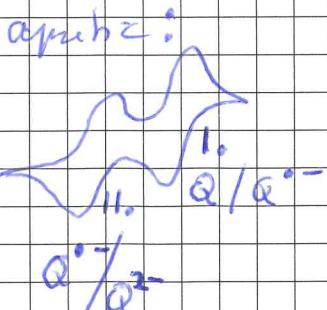
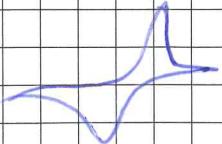
Coenzyme Q<sub>1</sub>

decylubiquinone

Coenzyme Q<sub>10</sub>

apotic  $\rightarrow$  non-proton donating solvent

apotic solvent:



- authors: SVW  $\rightarrow$  SWV
- activity instead of conc.  
temp?

To Page No. \_\_\_\_\_

Witnessed and understood by me

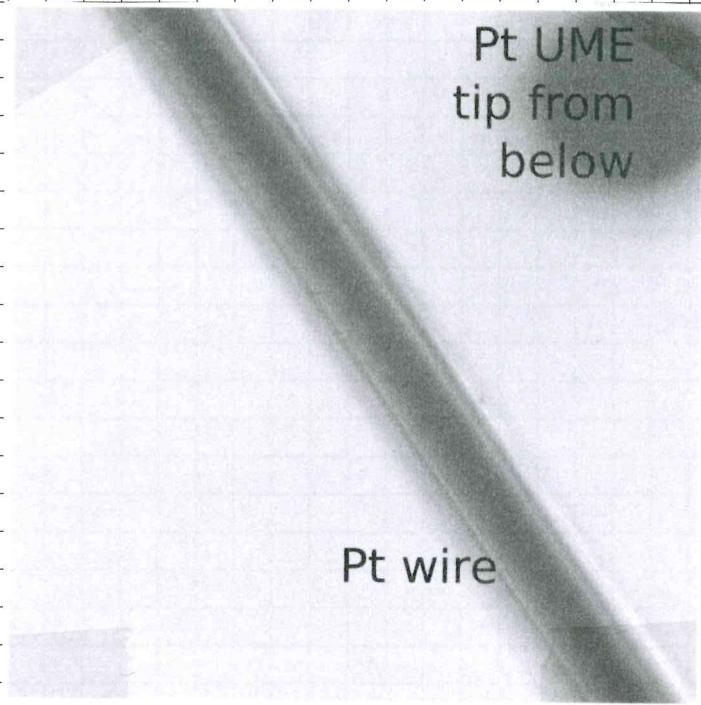
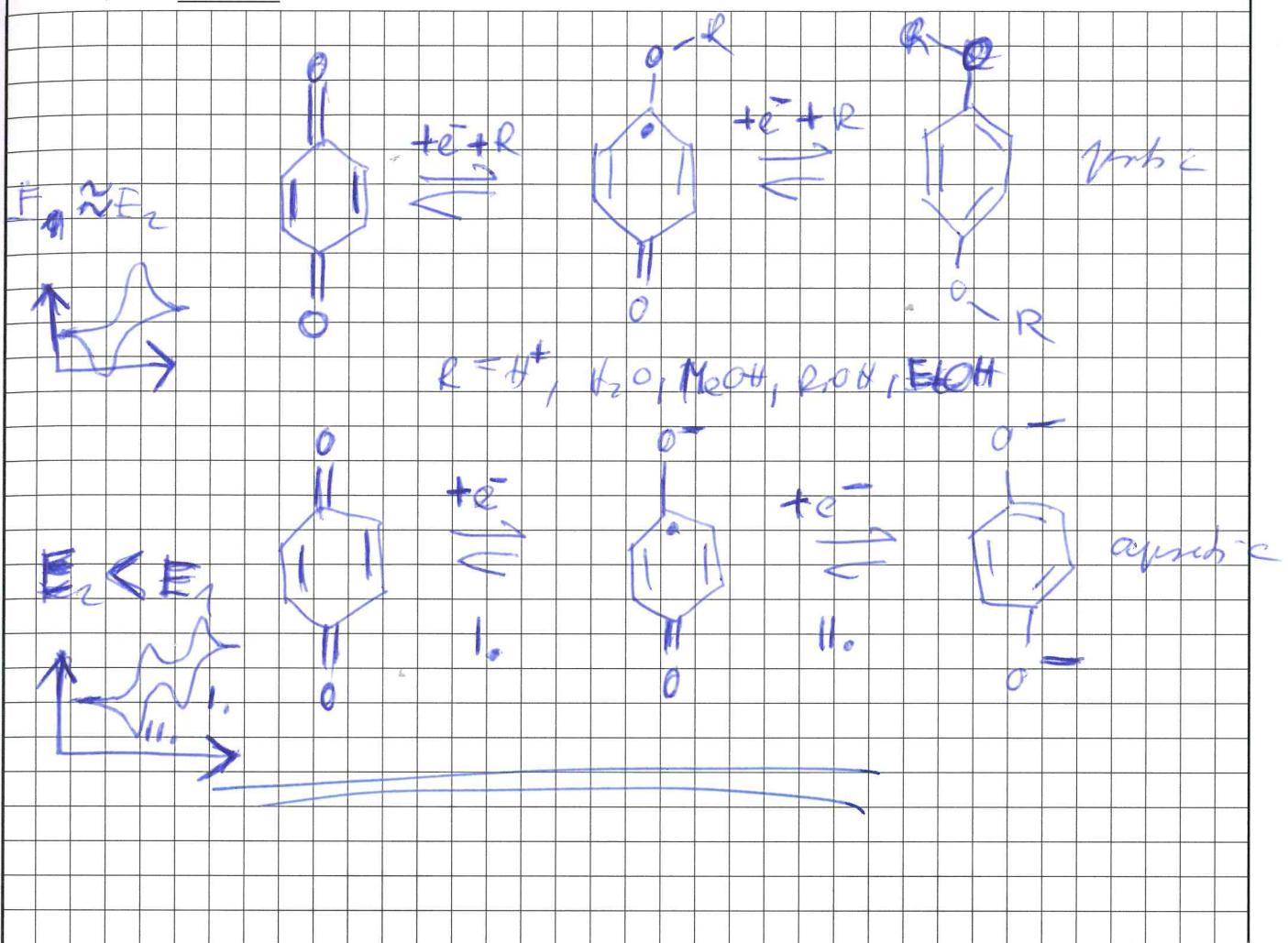
Date

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From Page No. \_\_\_\_\_



referred to on  
page 19

Pt wire

To Page No. \_\_\_\_\_

Witnessed and understood by me

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Date

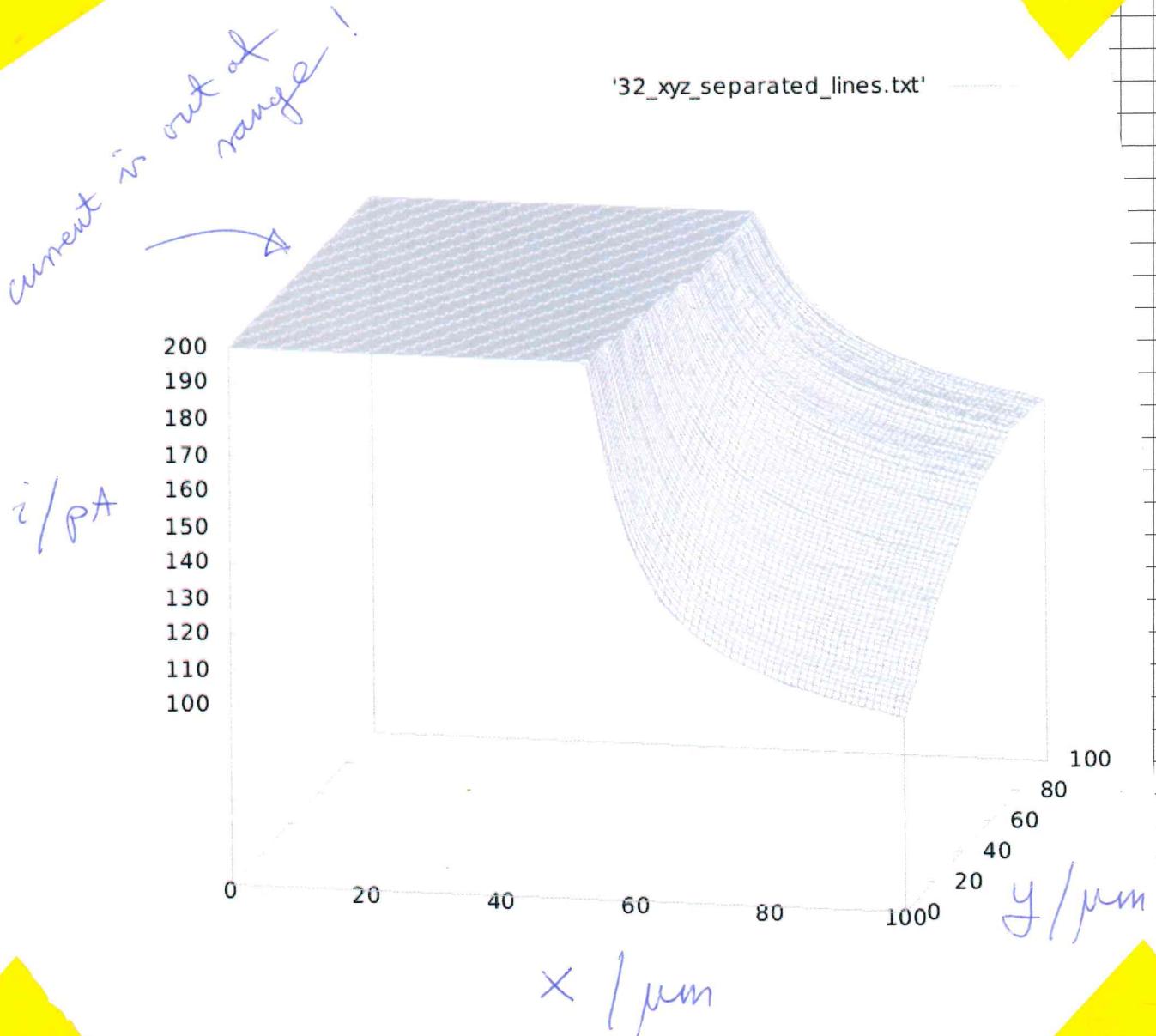
Recorded by

TITLE \_\_\_\_\_

Book No. \_\_\_\_\_

From Page No. \_\_\_\_\_

'32\_xyz\_separated\_lines.txt'



All of the scans from 180628 are clipped at ~200  $\mu\text{A}$ !

To Page No. \_\_\_\_\_

Witnessed and understood by me

Date

Invented by

Date

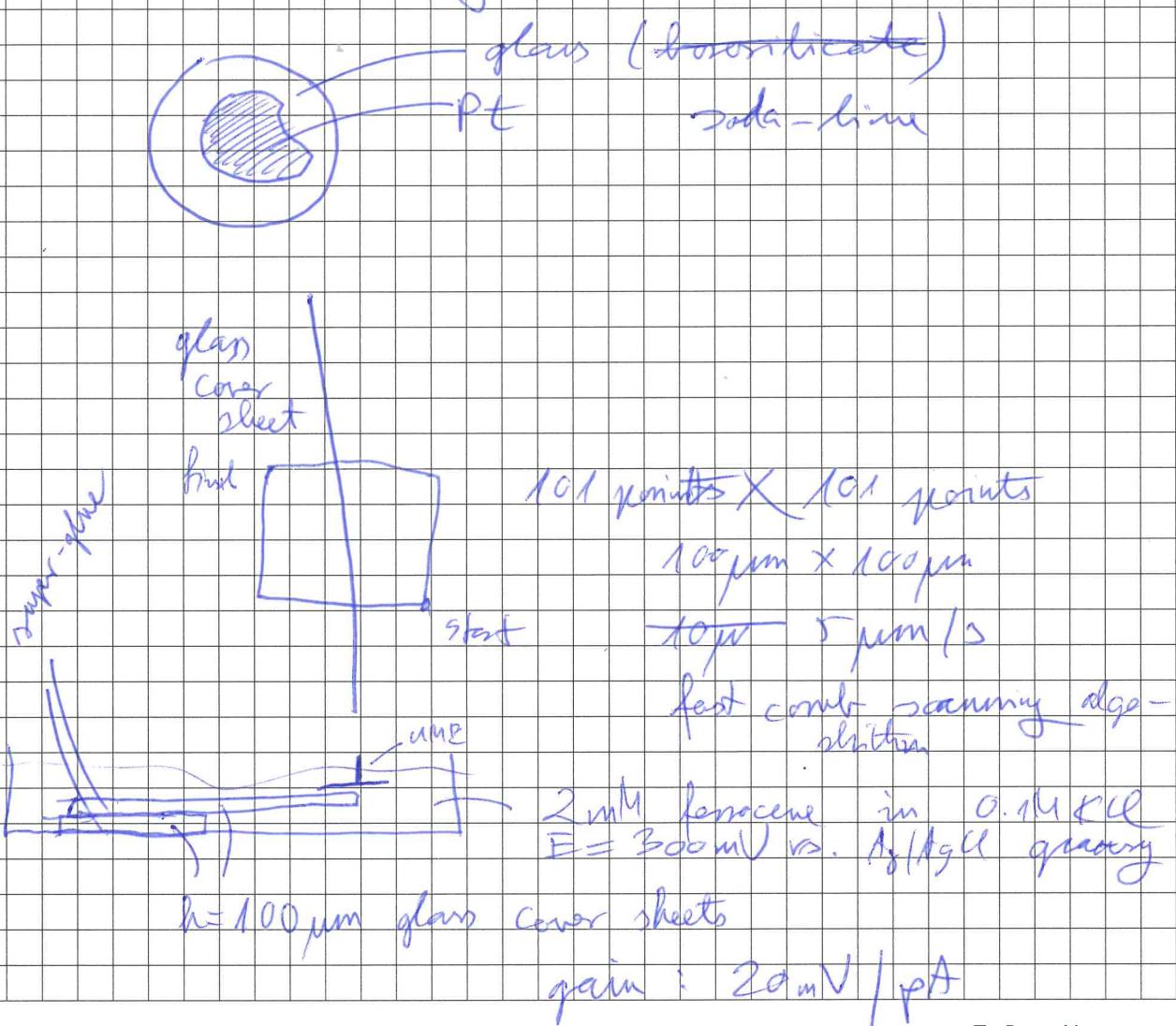
Recorded by

TITLE Soda-lime glass pulling

From Page No. \_\_\_\_\_

Sutter Instrument P-1000

Program 88

180704. Scanning with electrode #8 prepared  
yesterday

To Page No. \_\_\_\_\_

Witnessed and understood by me

Date

Invented by

Date

Recorded by

continued on next page

TITLE \_\_\_\_\_

From Page No. \_\_\_\_\_

continued from previous page

- 180704/E1-1

joints: cell 180704-003.TIF

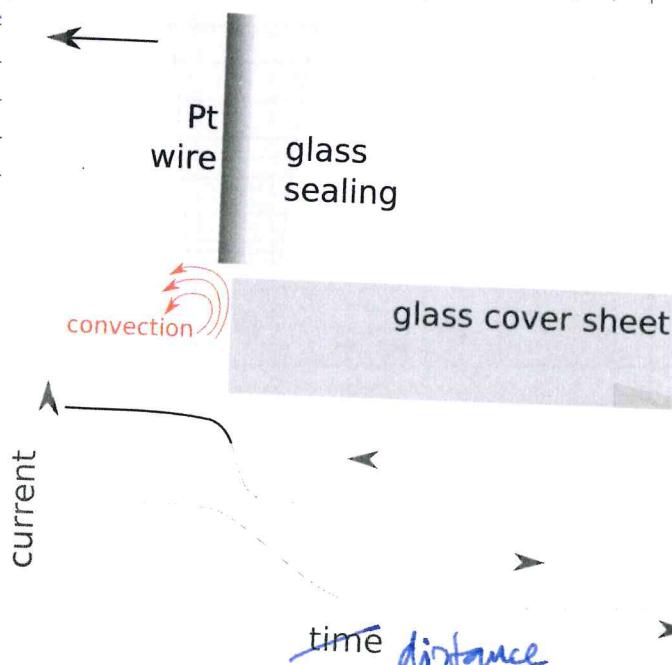
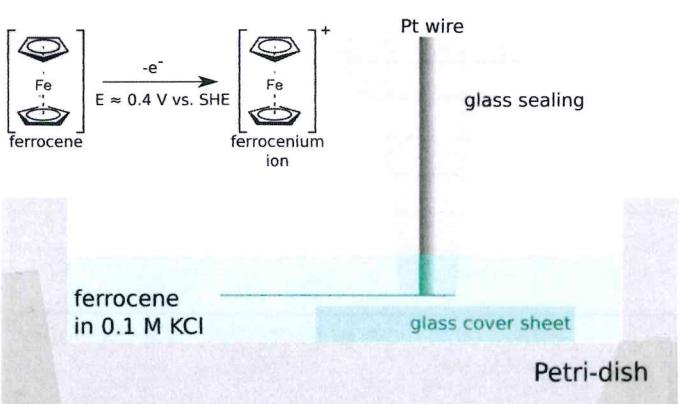
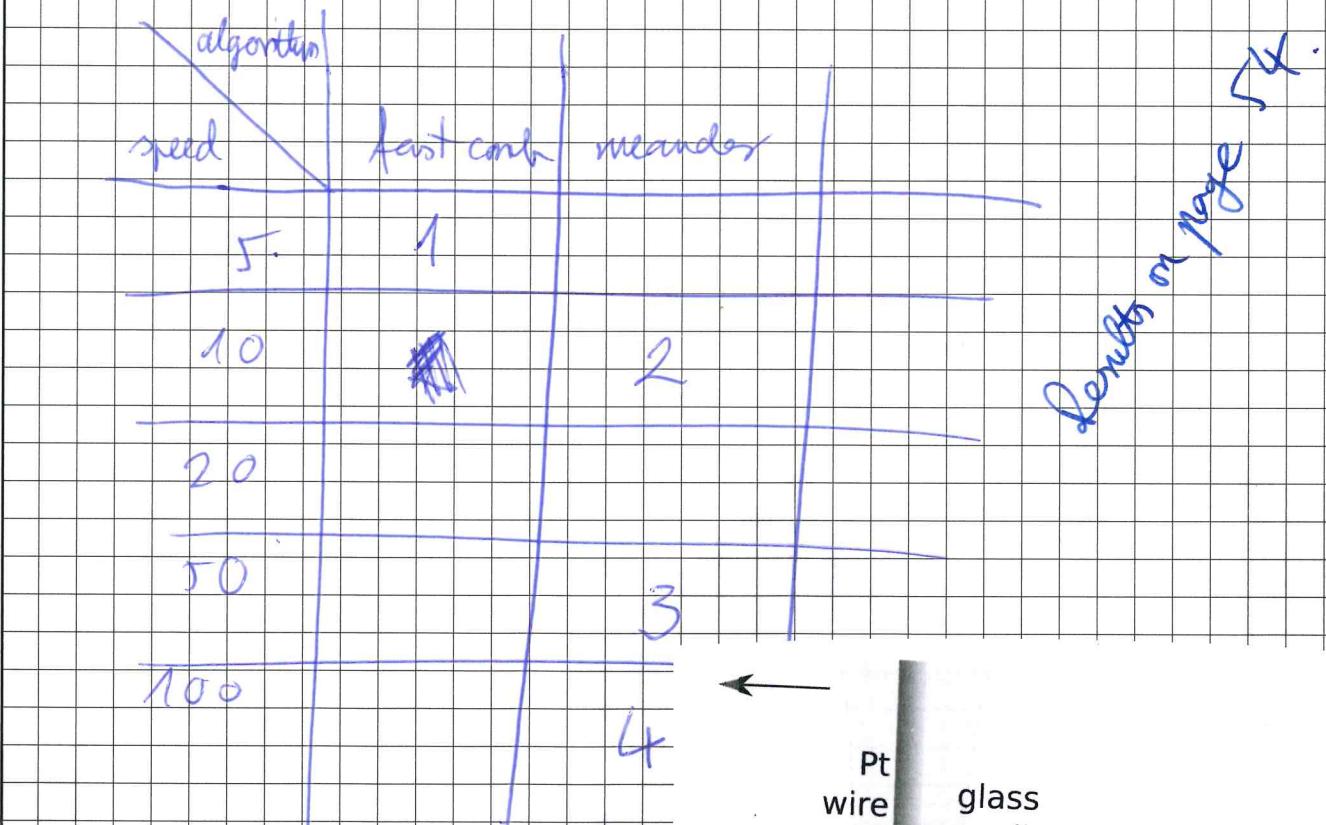
 $101 \times 101$   
 $10 \times 10$ 5  $\mu\text{m}/\text{s}$ 

fast comb

- 18070406/E1-2

10  $\mu\text{m}/\text{s}$ 

meander



TITLE Autoway microelectrode tests

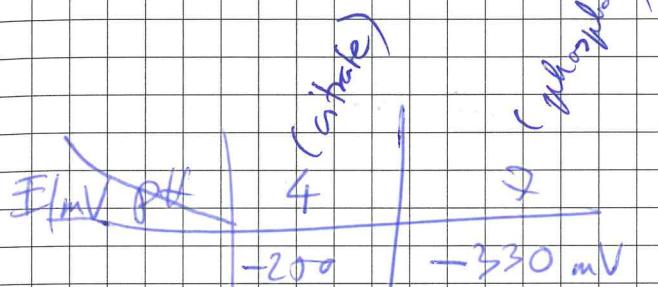
Book No. \_\_\_\_\_

From Page No. \_\_\_\_\_

Normal instruments pH meter

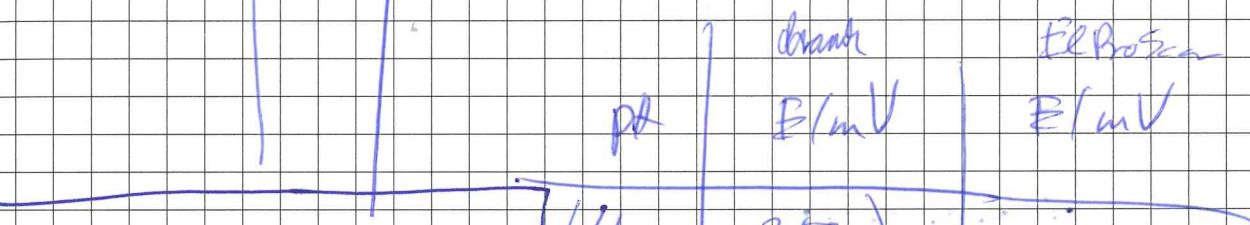
pH 211

microscrew pH meter



80mV / 3

26 mV / pH



Buffers were kindly provided by Katerina.

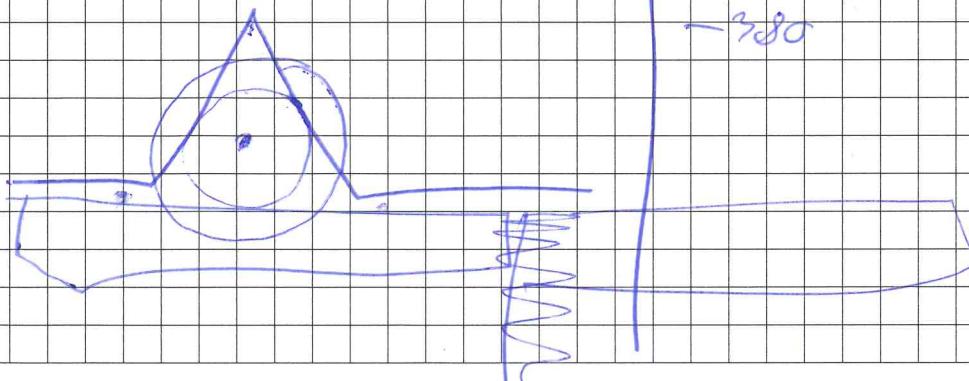
(4)  
7  
7  
4

-210  
-330  
-377  
-260

-368  
117/13  
39mV/pH

4

-262  
-380



To Page No. \_\_\_\_\_

Witnessed and understood by me

Date

Invented by

Date

Recorded by

TITLE Writing a script to fix the meander algorithm

From Page No. \_\_\_\_\_

Problem :

X Y Z

0	0	}
1	0	
2	0	
3	0	
4	0	
0	1	}
1	1	
2	1	
3	1	
4	1	

1st line

2nd line



The direction should be reversed for the even numbered lines, like this:

X Y Z

0	0	
1	0	
2	0	
3	0	
4	0	
4	1	
3	1	
2	1	
1	1	
0	1	

To Page No. \_\_\_\_\_

Witnessed and understood by me

Date

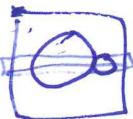
Invented by

Recorded by

Date

180705

HECA



Project No. \_\_\_\_\_

TITLE \_\_\_\_\_

Book No. \_\_\_\_\_

From Page No. \_\_\_\_\_

fc2m.sh "Fast curl to meander"

inputs : 1) # of ~~point~~ points in a line (x)

example from the left: x=5

2) # of lines (r)

example from the left: y=2

usage: fc2m -x 5 -y 2 -f test.txt  
-o test-o.txt

bash arguments: getopt

```
while getopt u:d:p:f: option
do
case "$OPTARG" in
in
u) USR=$OPTARG;;
d) DSE=$OPTARG;;
p) PRT=$OPTARG;;
f) FNC=$OPTARG;;
:
esac
done
```

→ finished script on next page!

To Page No. \_\_\_\_\_

Witnessed and understood by me

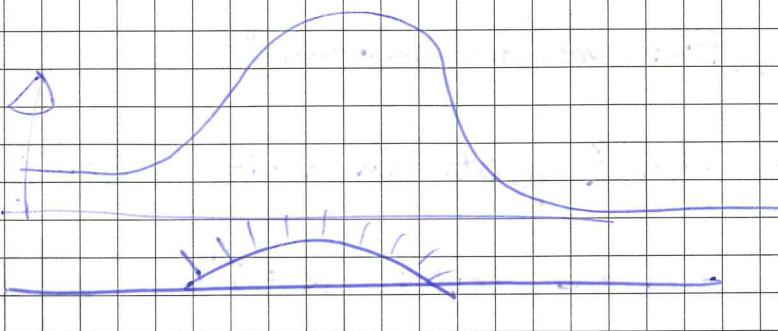
Date

Invented by

Date

Recorded by

From Page No. \_\_\_\_\_



```

#!/bin/bash

POSITIONAL=()
while [[ $# -gt 0 ]]
do
key="$1"

case $key in
-x|--x)
x="$2"
shift # past argument
shift # past value
;;
-y|--y)
y="$2"
shift # past argument
shift # past value
;;
-if|--inputfile)
inputfile="$2"
shift # past argument
shift # past value
;;
-of|--outputfile)
outputfile="$2"
shift # past argument
shift # past value
;;
*)      # unknown option
POSITIONAL+=("$1") # save it in an array for later
shift # past argument
;;
esac
done
set -- "${POSITIONAL[@]}" # restore positional parameters

#old version done with sed, not complete
#cp /dev/null $outputfile
#for i in $(seq 0 2 $y); do
# sed -n "$((i*x+1)),${((i*x+x))} p" "$inputfile" >> "$outputfile"
# sed -n "$(((i+1)*x+1)),${((i+1)*x+x))} p" "$inputfile" | tac >> "$outputfile"
#done

#with awk
cp /dev/null $outputfile
for i in $(seq 0 2 $y); do
awk -v line="$i" "NR>=${((i*x+1))} && NR<=${((i*x+x))}" '{print $2*1000000, line, $3*1000000000000}' \
$inputfile >> $outputfile
awk -v line="$i" "NR>=${((i+1)*x+1))} && NR<=${((i+1)*x+x))}" '{print $2*1000000, line+1,
$3*1000000000000}' "$inputfile" | tac >> "$outputfile"
done

```

To Page No. \_\_\_\_\_

Witnessed and understood by me	Date	Invented by	Date
		Recorded by	

TITLE Trying out a new technique to fabricate SB microelectrodes

From Page No. \_\_\_\_\_

~~antimony powder~~ Borosilicate glass

P-1000 borosil glass

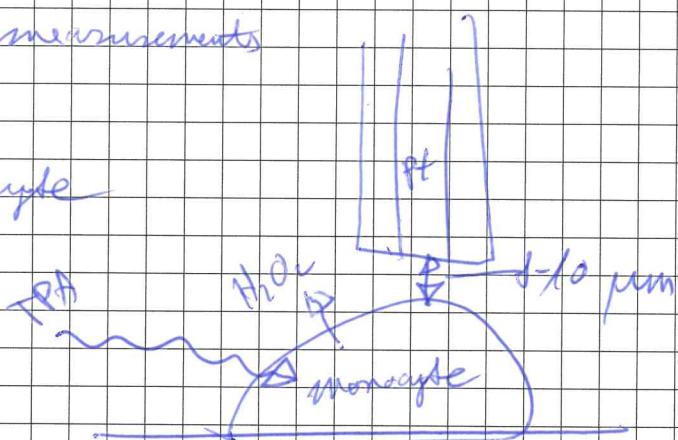
180210 | H<sub>2</sub>O<sub>2</sub> measurements

above monocyte

$$E = 650 \text{ mV}$$

$$50 \mu\text{m} / 50 \mu\text{m}$$

$$2 \mu\text{m} / 2 \mu\text{m}$$



180210-1. dat

1. 3D-scan: fast scan 5 μm/h

2. area - measure 10 μm/h

plate mixed

To Page No. \_\_\_\_\_

Witnessed and understood by me

Date

Invented by

Date

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TITLE Testing antimony microspherules prepared yesterday

Book No. \_\_\_\_\_

From Page No. \_\_\_\_\_

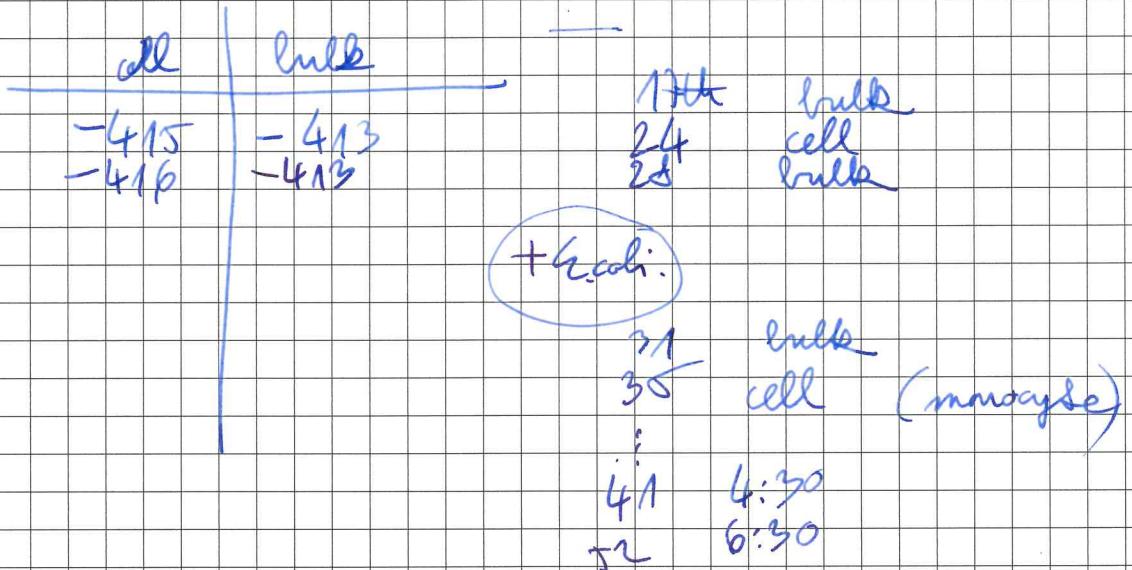
$E@ pH=7 PBS$  (mV)

- |    |         |
|----|---------|
| 1  | —       |
| 2  | -403.16 |
| 3  | -394    |
| 4  | -378    |
| T  | -368    |
| 6  | -350    |
| 7  | -350    |
| 8  | -350    |
| 9  | -358    |
| 10 | -357    |

9/10



62 pixel = 10  $\mu\text{m}$  (40X)



$-430\text{mV}$   $\xrightarrow{300\mu\text{l}}$   $-696\text{mV}$   
1N NaOH

To Page No. \_\_\_\_\_

Witnessed and understood by me

Date

Invented by

Date

Recorded by

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE Attempting to image yeast cell CO<sub>2</sub> output

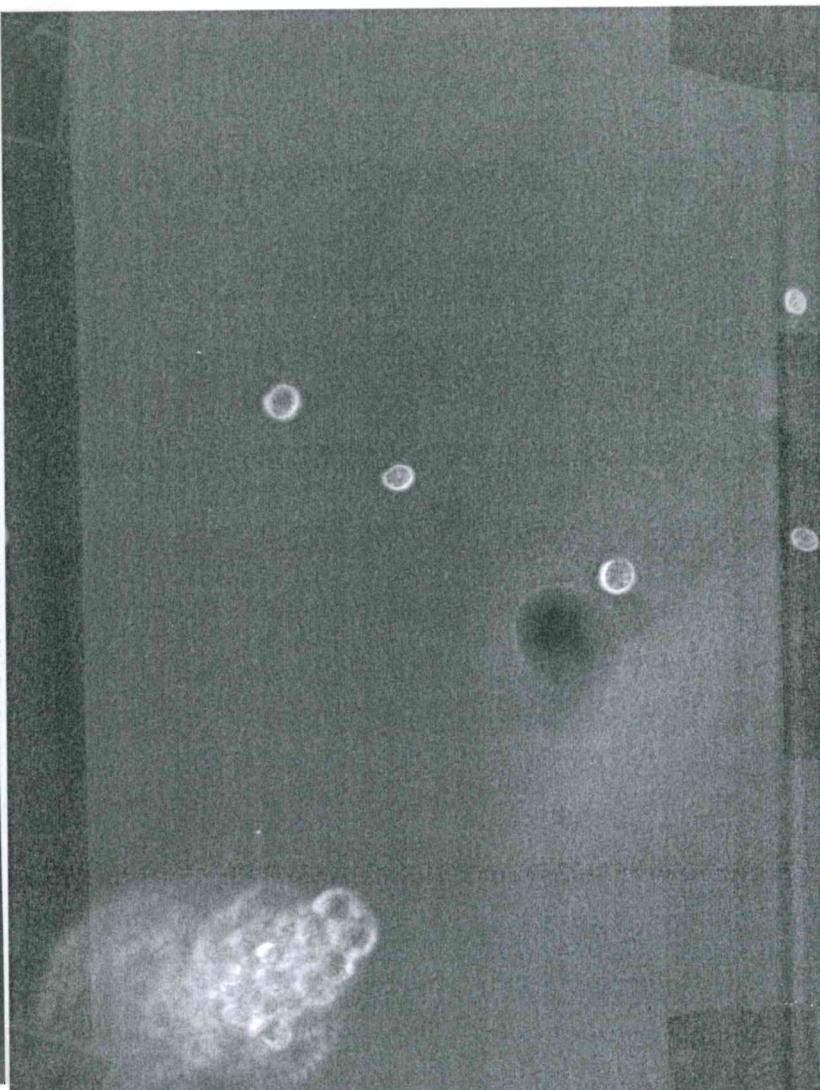
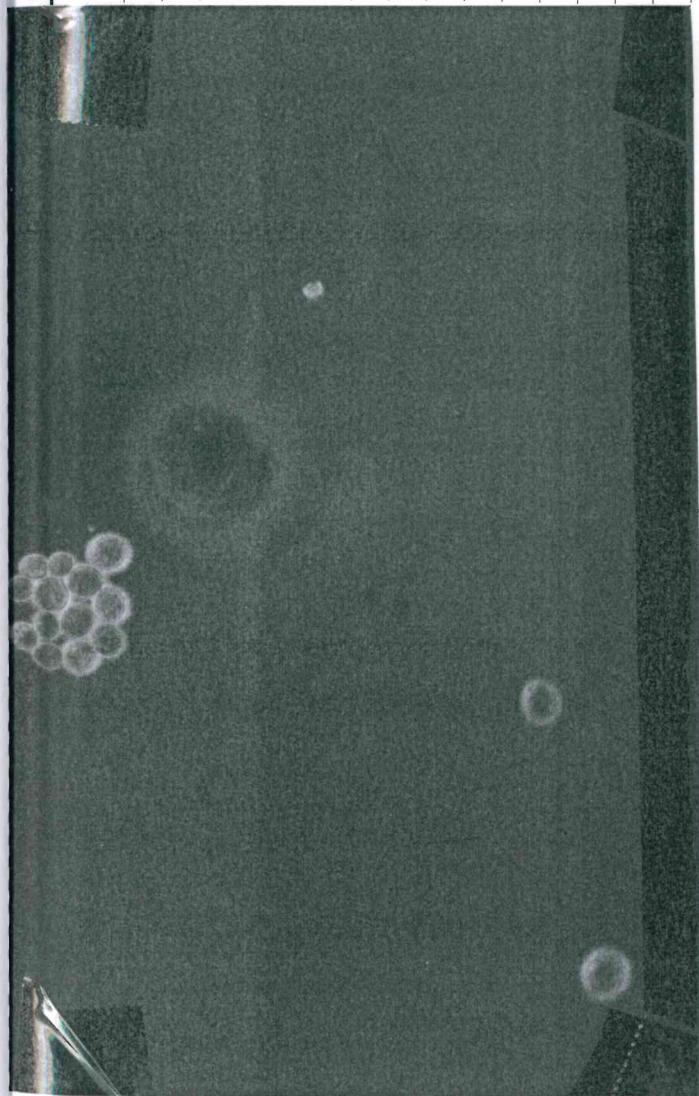
From Page No. \_\_\_\_\_

Broth : 2g glucose in 100 ml d.w.

Yeast : "Oma's Ur-Hefe Universal" from Edeka

I could not observe any pH change above  
the yeast cells.

(Pt electrode #7 broke.)



.....and understood by me

Date

Recorded by

Date

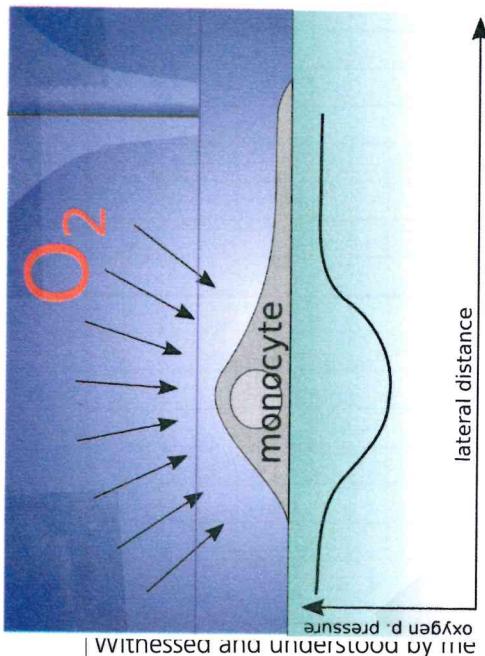
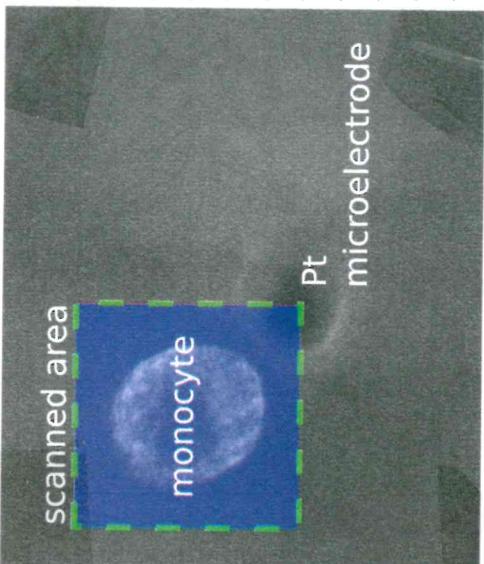
TITLE Masuring oxygen above the human monocytes

From Page No. \_\_\_\_\_

180716-01

E1 - ?

incomplete



Monocytes 6 days old

$100\mu\text{m} \times 100\mu\text{m}$  area  
 $101 \times 101$  points



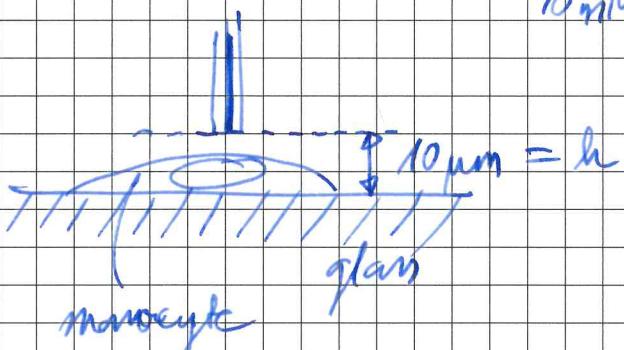
$1\mu\text{m} \times 1\mu\text{m}$  step size

$10\mu\text{m}/\text{s}$  scanning speed  
meander algorithm  
electrode #8



$E = -700\text{mV}$  vs. quasi-reference  
(dissolved Miller wire)

medium + electrolyte: PBS  
10 mM glucose



To Page No. \_\_\_\_\_

all by a witnessed and witnessed under and understood

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180716

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Date

TITLE \_\_\_\_\_

Book No. \_\_\_\_\_

From Page No. \_\_\_\_\_

E1 - 3

 $40\text{ }\mu\text{m} / 40\text{ }\mu\text{m}$  $41 \times 41$  $5\text{ }\mu\text{m/s}$  meander $e^{-t}$ 

incomplete

start: 3

finish: 4

(Cell 180716\_003.TIF)

E1 - 4

 $40\text{ }\mu\text{m} \times 40\text{ }\mu\text{m}$  $41 \times 41$ 

incomplete

 $1\text{ }\mu\text{m/s}$  fast comb

E1 - 5

 $40\text{ }\mu\text{m} \times 40\text{ }\mu\text{m}$  $41 \times 41$  $h = 3.15\text{ }\mu\text{m}$  $10\text{ }\mu\text{m/s}$  meander

E2 - 1

 $60\text{ }\mu\text{m} \times 60\text{ }\mu\text{m}$  $61 \times 61$  $0.7\text{ }\mu\text{m/s}$  fast comb

new cell

 $z = -26.71\text{ }\mu\text{m}$ 

E2 - 8

 $x \quad y$  $40 \times 40$  $801 \times 41$ 

fast comb

 $1\text{ }\mu\text{m/s}$  $40 \times 40$  $401 \times 41$ 

meander

 $10\text{ }\mu\text{m/s}$ E2 - 10 - 11 -  
 $20\text{ }\mu\text{m/s}$  meander

E2 - 9

Only  $\rightarrow 401$ , because it's 10 times faster

To Page No. \_\_\_\_\_

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HEKA

E2 - 11 - v

 $5\text{ }\mu\text{m/s}$   
meanderE2 - 12 - v  
 $0.5\text{ }\mu\text{m/s}$   
fast comb

TITLE continued from previous page

From Page No.

$v(\mu\text{m/s})$  fast comb meander

1 (E2-13)

5 E2-8

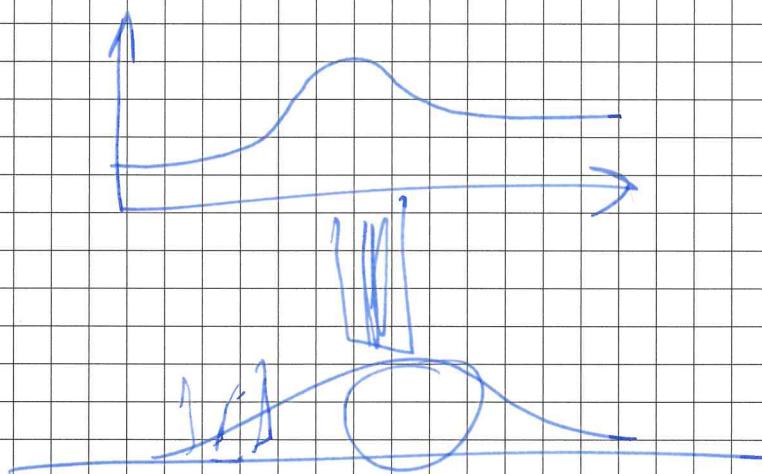
10 E2-9

20 E2-10

50 E2-11

+ step response? 

cell dead: E2-15



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 $+ 20 \mu\text{l} \text{ of } 100\text{mM H}_2\text{O}_2$ 

$$V_{\text{total}} = 2 \text{ ml}$$

$$20 \cdot 10^{-6} \text{ dm}^3 \cdot 0.1 \frac{\text{mol}}{\text{dm}^3}$$

~~$2 \cdot 10^{-5}$~~

$2 \cdot 10^{-5} \text{ dm}^3$

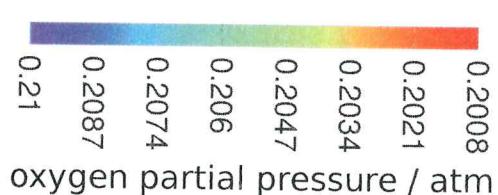
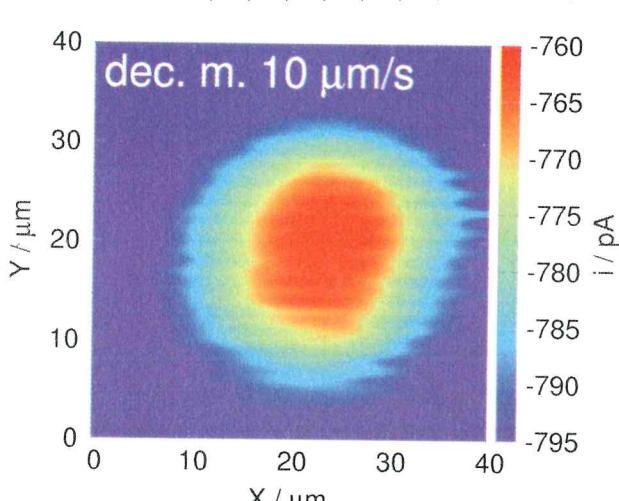
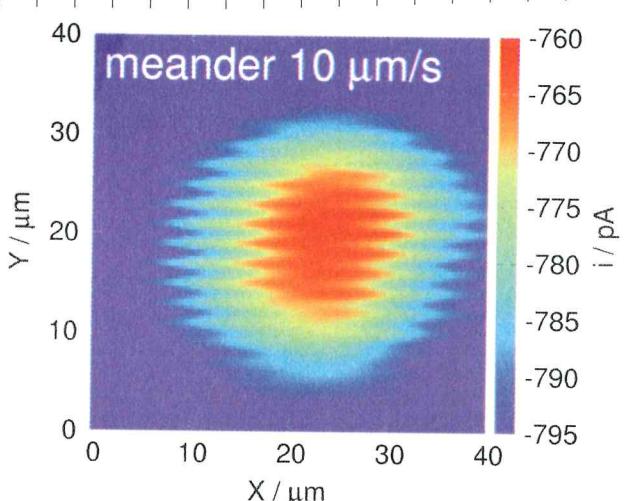
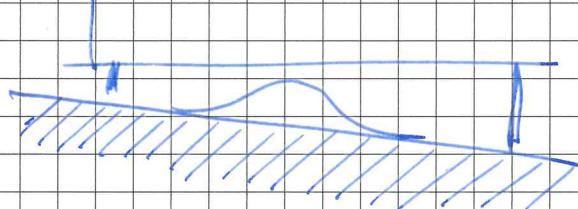
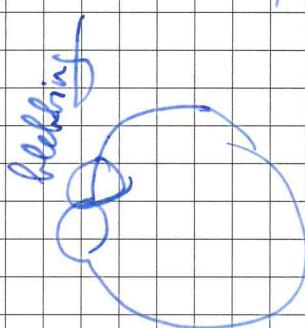
$2 \cdot 10^{-8} \text{ mol}$

$2 \mu\text{mol} \text{ in } 0.002 \text{ dm}^3$

$c = \frac{0.00002 \text{ mol}}{0.002 \text{ dm}^3}$

 $\downarrow$ 

$c = 1 \text{ mM H}_2\text{O}_2$



E2-9

From Page No. \_\_\_\_\_

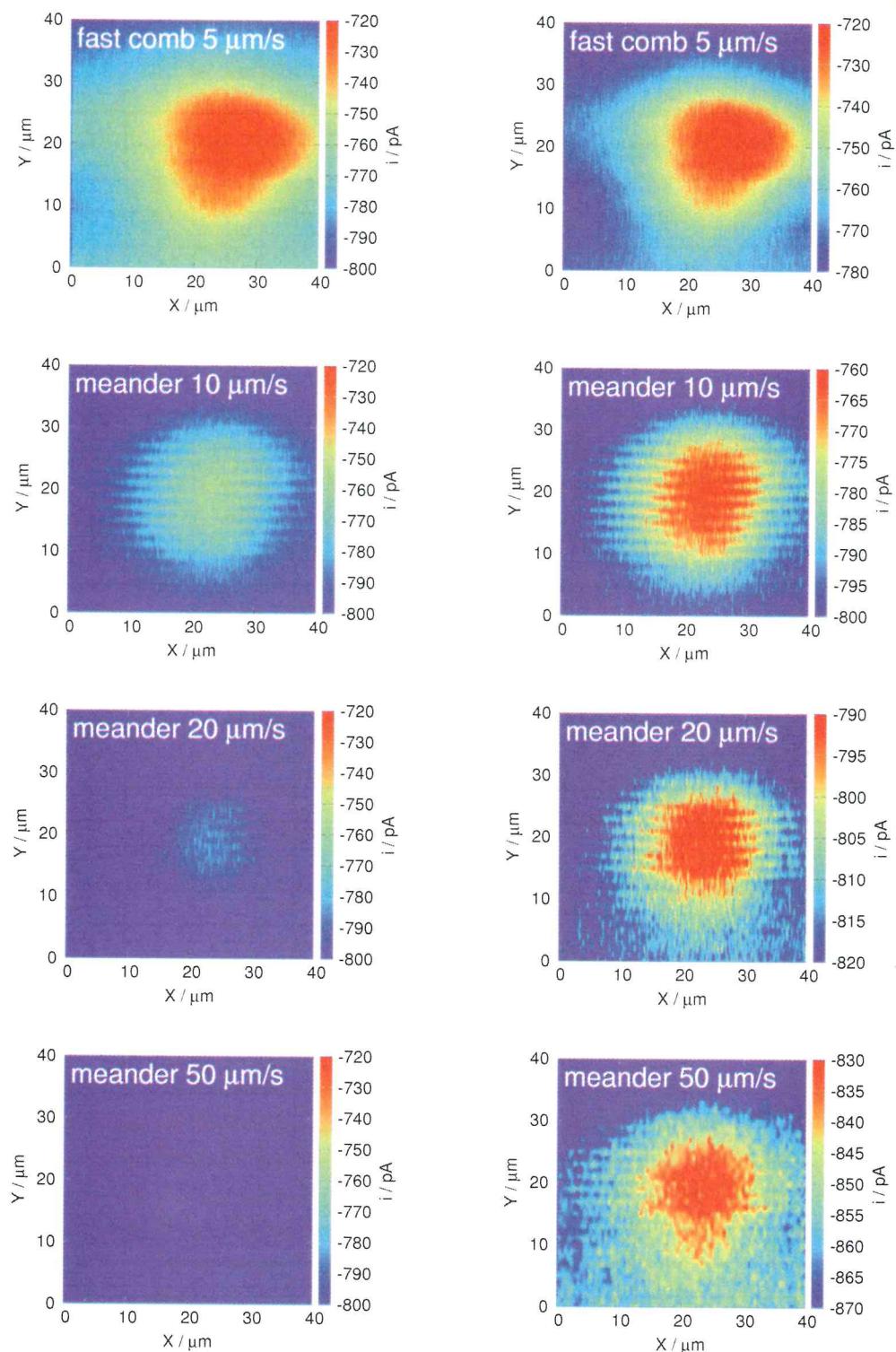


Figure 1: Oxygen reduction current above a human monocyte at  $h = 10 \mu\text{m}$  relative to the glass bottom of the Petri-dish. Working electrode:  $d = 10 \mu\text{m}$  Pt UME.  $\text{RG} \approx 2.5$ .  $E = -700 \text{ mV}$  vs. Ag/AgCl quasi-reference electrode. Medium/electrolyte: PBS + 10 mM glucose. Date: 2018.07.16. Left column: fixed scale -800 pA to -720 pA. Right column: autoscale.

10 page in.

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Date \_\_\_\_\_

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Recorded by \_\_\_\_\_

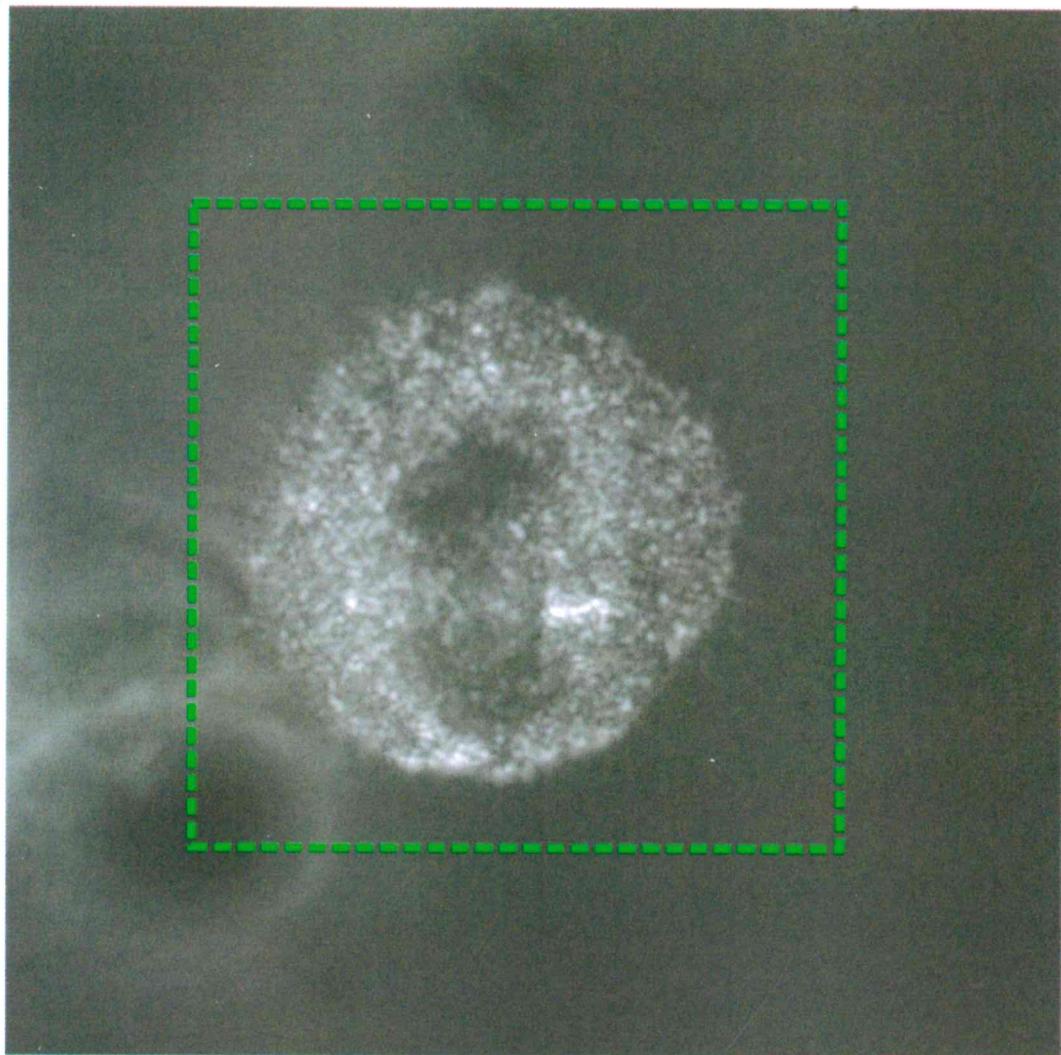
Date \_\_\_\_\_

Project No. \_\_\_\_\_

TITLE \_\_\_\_\_

Book No. \_\_\_\_\_

From Page No. \_\_\_\_\_



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Date

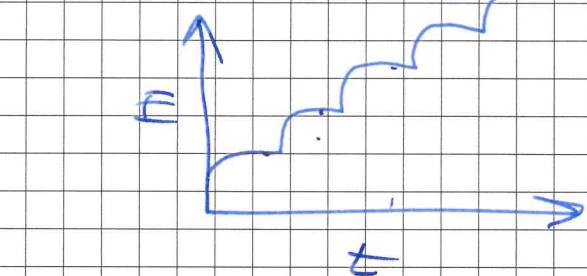
Recorded by

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1, Create pH = 6, 6.5, 7, 7.5 & 8.5 of L-buffer  
 ↓ (PPS) ↓ ↓ ↓ ↓  
 real pH 6.06 6.17 7.06 7.5 8.03 8.42  
 8.42  
 adjusted with 1N HCl and 1N NaOH

measured with Hanna pH 211

pH	E/mV
6.06	-293.1
6.57	-318
7.06	-344
7.5	-364
8.03	-388
8.42	-403



D. Mebohm

Ag/AgCl/3M KCl  
ref. electrode

with HEKA  
patch-clamp

EPC 10 USB

To Page No. \_\_\_\_\_

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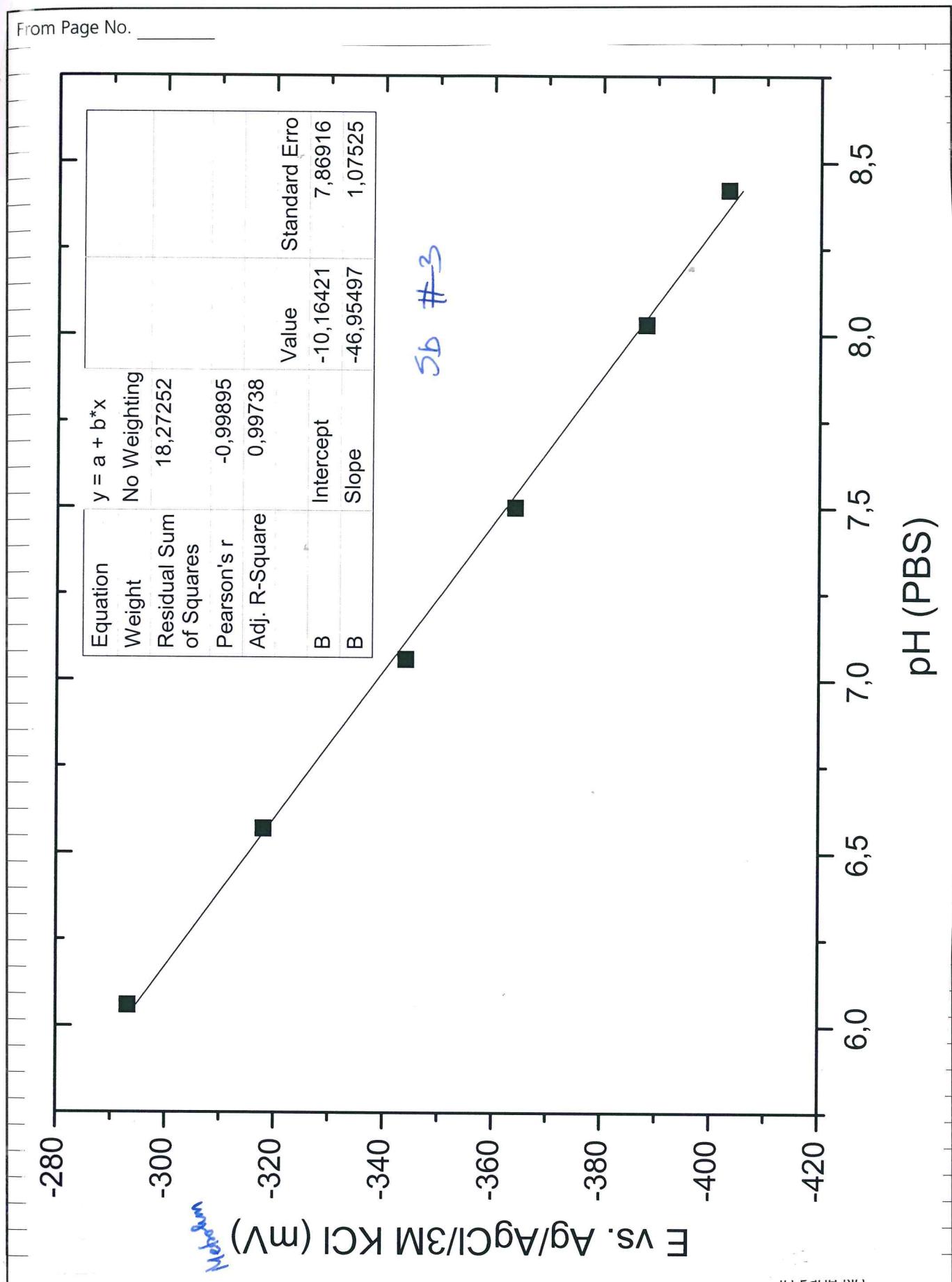
Invented by

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$\text{pH}$	$E(\text{mV})$ vs $\text{Ag}/\text{AgCl}$ quan-xf. (chlorinated silver wire)
6.06	-315
6.57	-336
7.06	-318
7.5	-377
8.03	-397
8.42	-415

The slope is similar to that of  $V$  measured with an  $\text{Ag}/\text{AgCl}/\text{3M KCl}$  reference electrode.

The potentials are shifted by about  $-20\text{mV}$ , as a consequence of the shift in the potential at the reference half-cell.

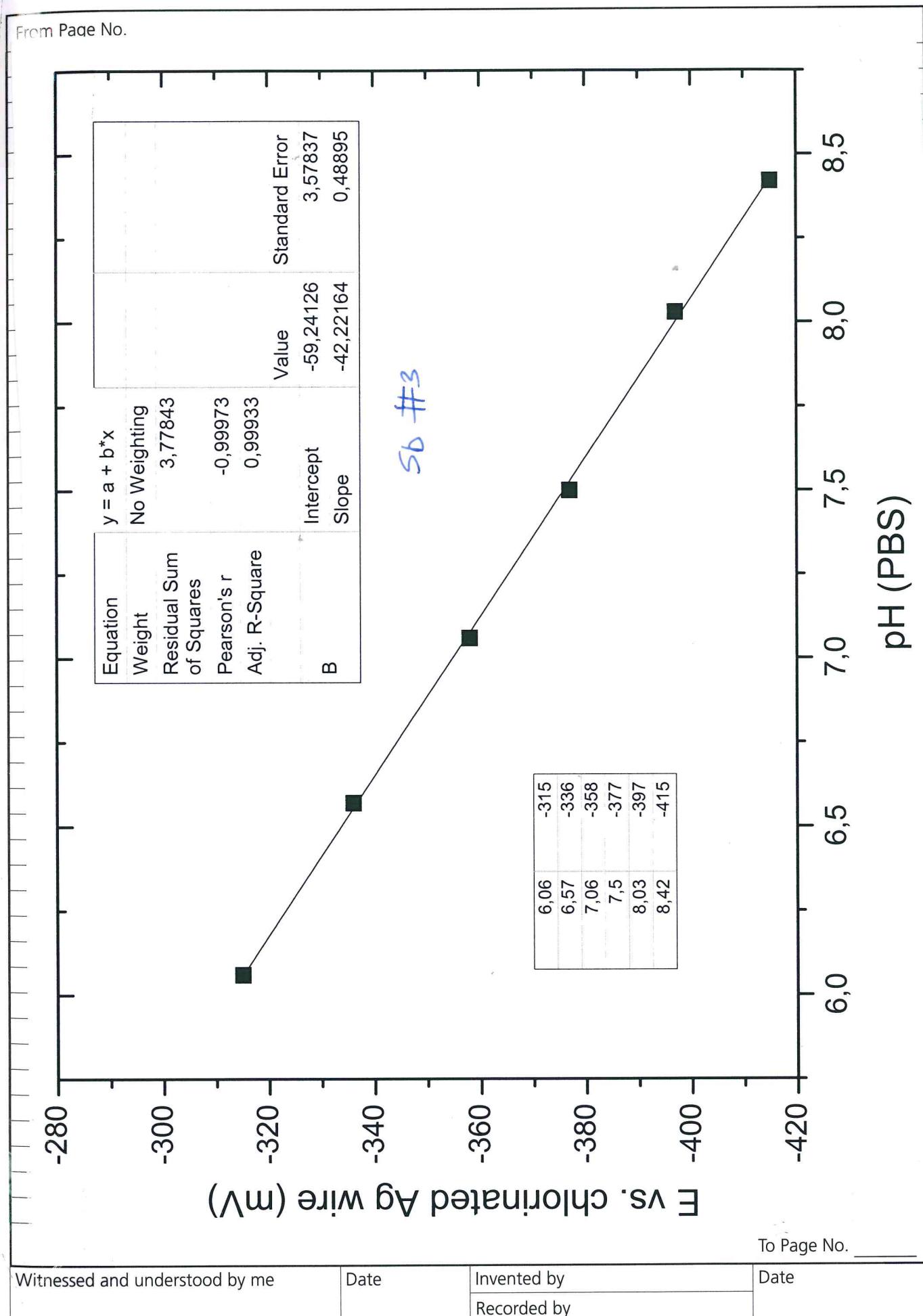
The minor difference in  $\text{aCe}^-$  of the buffers doesn't seem to effect the response noticeably.

To Page No. \_\_\_\_\_

Witnessed and understood by me	Date	Invented by	Date
		Recorded by	

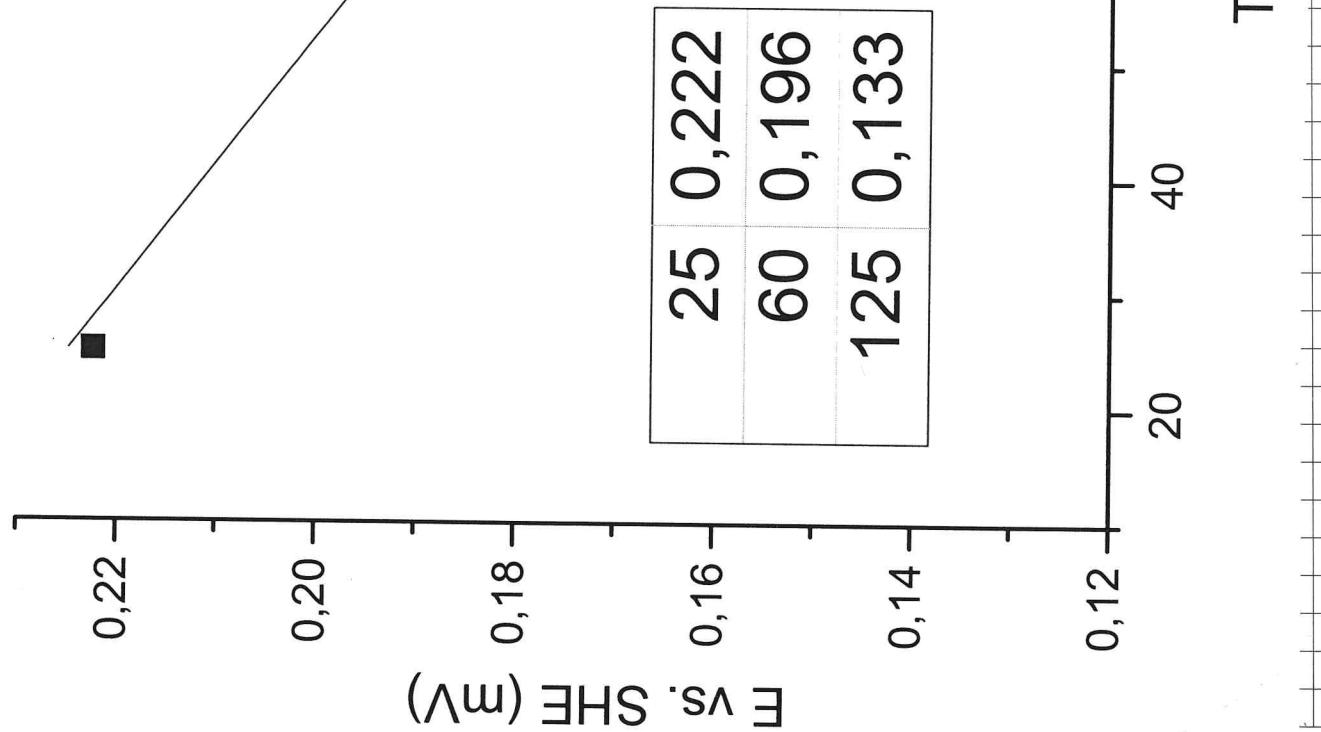
TITLE \_\_\_\_\_

From Page No. \_\_\_\_\_



From Page No.

Equation	$y = a + b*x$
Weight	No Weighting
Residual Sum of Squares	2,12919E-5
Pearson's r	-0,99748
Adj. R-Square	0,98994
B	
Intercept	0,24735
Slope	-9,04437E-4
Value	0,00523
Standard Error	6,429989E-5



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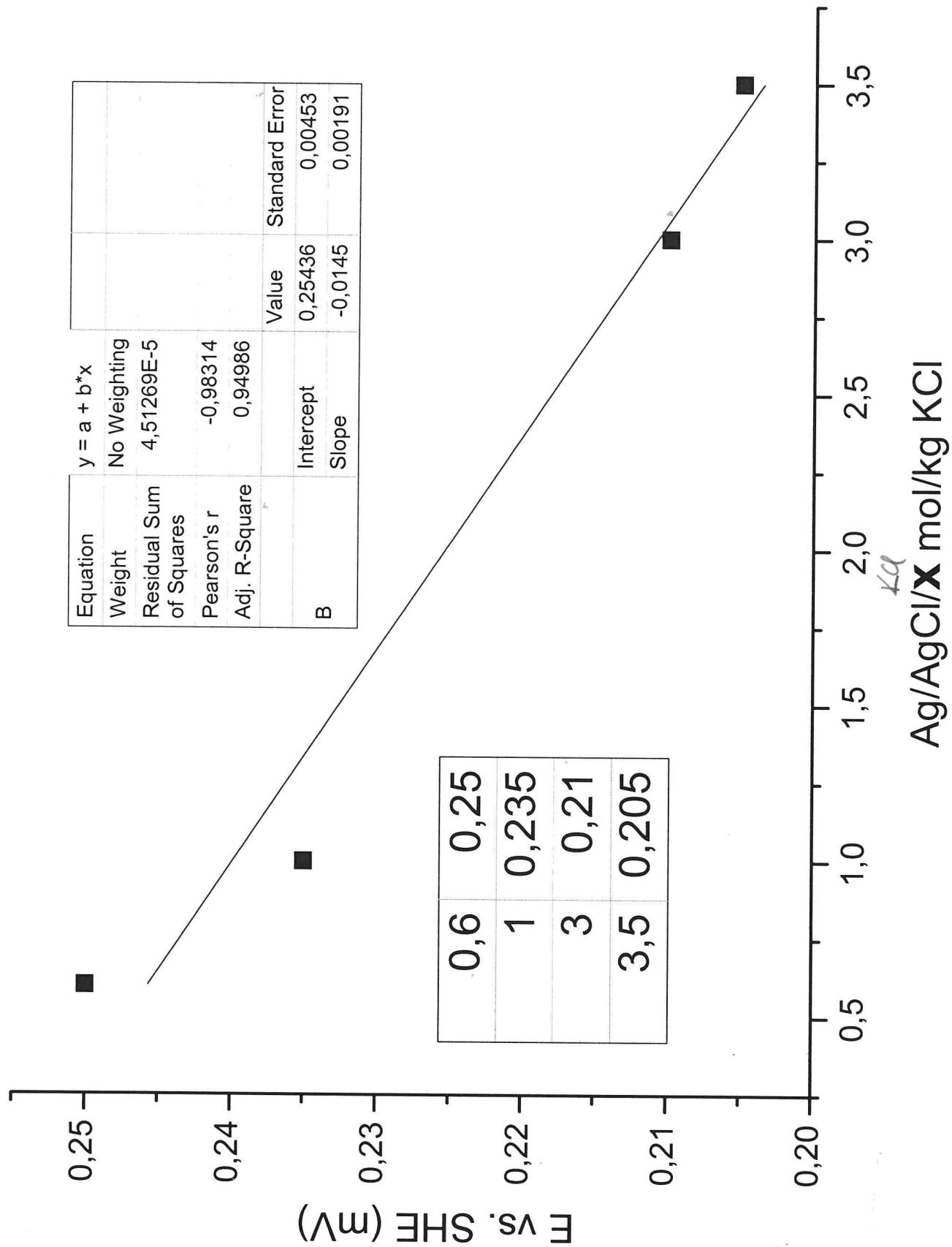
Project No. \_\_\_\_\_

TITLE \_\_\_\_\_

Book No. \_\_\_\_\_

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	$y = a + b \cdot x$
Weight	No Weighting
Residual Sum of Squares	4,51269E-5
Pearson's r	-0,98314
Adj. R-Square	0,94986
B	
Intercept	0,25436
Slope	-0,0145
	Value Standard Error
	0,00453 0,00191



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TITLE Investigating the noise problem

Book No. \_\_\_\_\_

From Page No. \_\_\_\_\_

The noise in chronoamperometric measurements increased as temperature increases.

2ml PBS

 $E = 650 \text{ mV}$ 

Pt UME # 8

 $t = 36.8^\circ\text{C}$ 

$$\{\text{noise} \approx 3.48 \text{ pA} - 3.53 \text{ pA} \approx 305 \text{ fA}$$

1:22:30 thermostat off

$t / ^\circ\text{C}$	$\approx \text{noise}$
36.8	305 fA
32.8	270 fA
31.3	200 fA
30.0	<del>150 fA</del> - 150 fA
28.0	130 fA
22.0	110 fA
26.0	90 fA
23.0	60

Arrhenius - equation

$$k = A e^{-\frac{E_a}{RT}}$$

(1:51:00 + ice  $\sim 10^\circ\text{C}$ )  
 (1:54:00 + ice)

$16^\circ\text{C}$	58 fA
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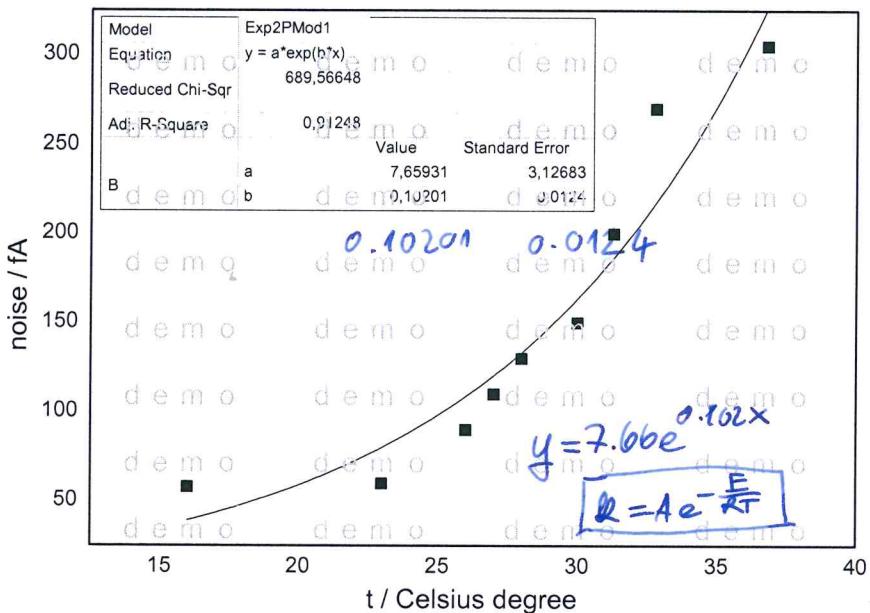
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180719

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From Page No. \_\_\_\_\_

MNN

The observed noise might be caused by the increased recombination rate. It appears that the magnitude of the noise follows the Arrhenius Form.

$$R = A e^{-\frac{E}{RT}}$$

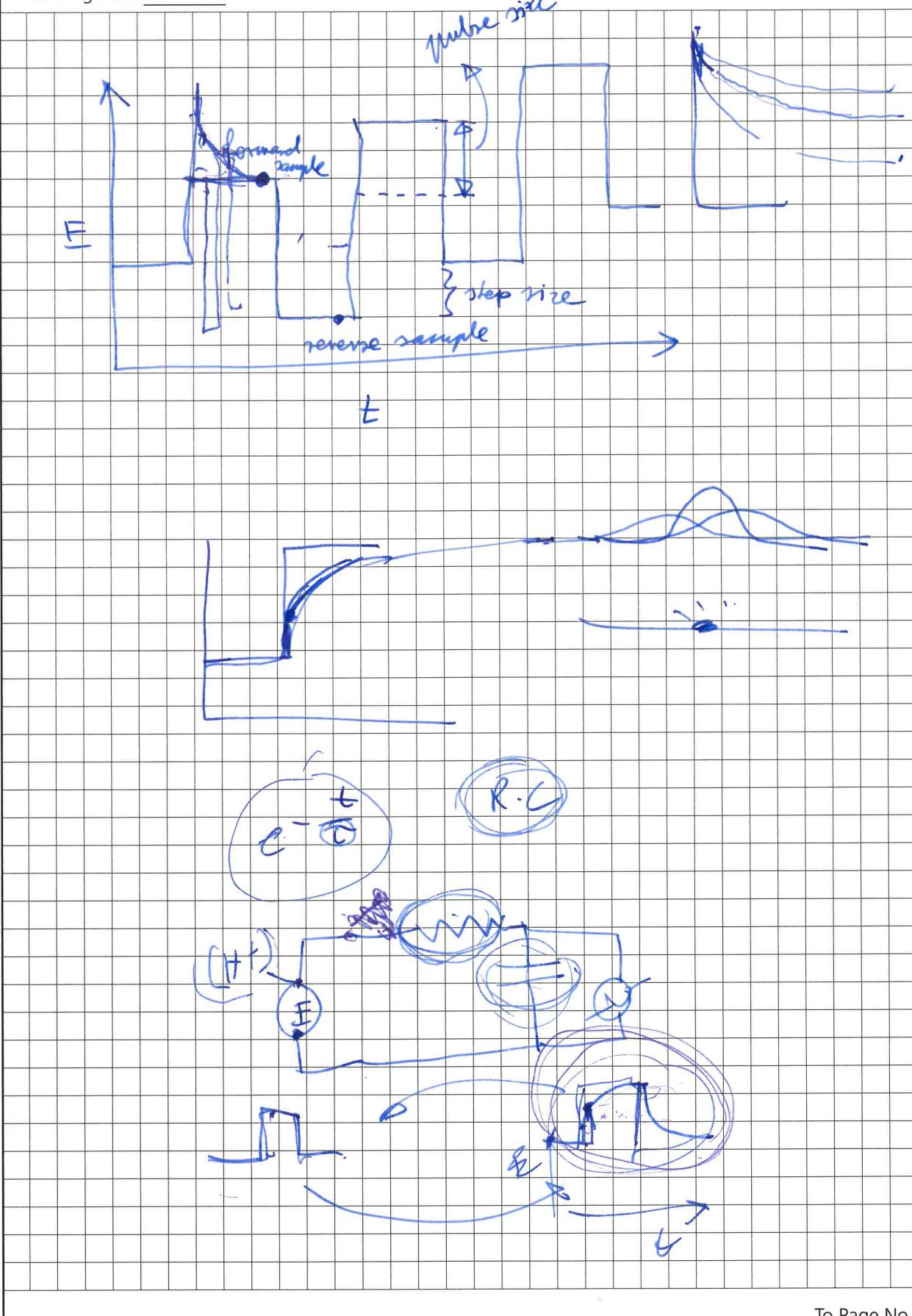
Note on 2010.08.07.:  
The signal is not increasing with temp., as recomb., because the rate limiting step is not the electrode reaction.  
It's transport limited.

To Page No. \_\_\_\_\_

TITLE Square wave voltammetry

(Diananin with Valenbin)

From Page No. \_\_\_\_\_



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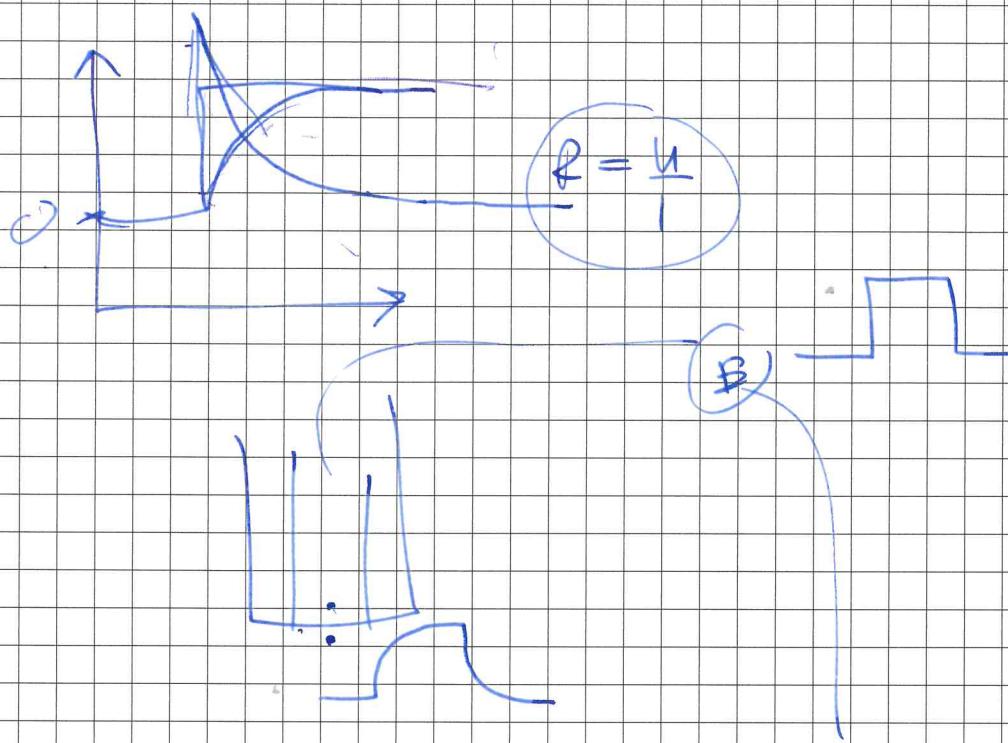
Date \_\_\_\_\_

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TITLE \_\_\_\_\_

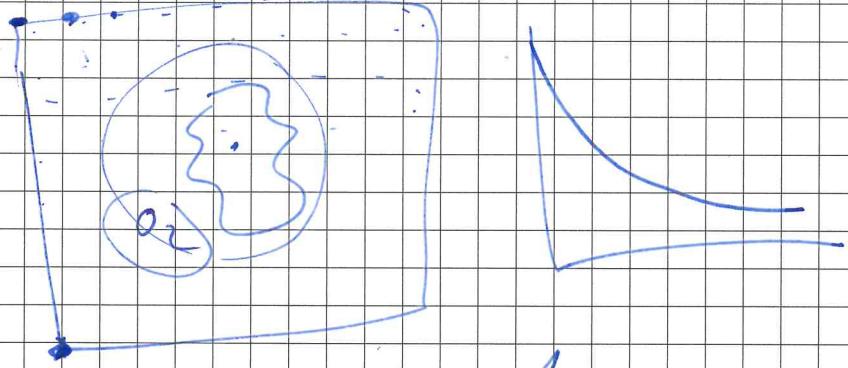
Book No. \_\_\_\_\_

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Discussion with Valentine

$$i_t = ae^{-\frac{t}{\tau}}$$

$$\frac{1}{\sqrt{t}} \rightarrow \infty$$



$$\frac{1}{\sqrt{t}}$$

$$y = \frac{1}{\sqrt{x}}$$

$$x = \left(\frac{1}{y}\right)^2$$

To Page No. \_\_\_\_\_

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Date

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TITLE Platinum etching in aqua regia

Book No. \_\_\_\_\_

3

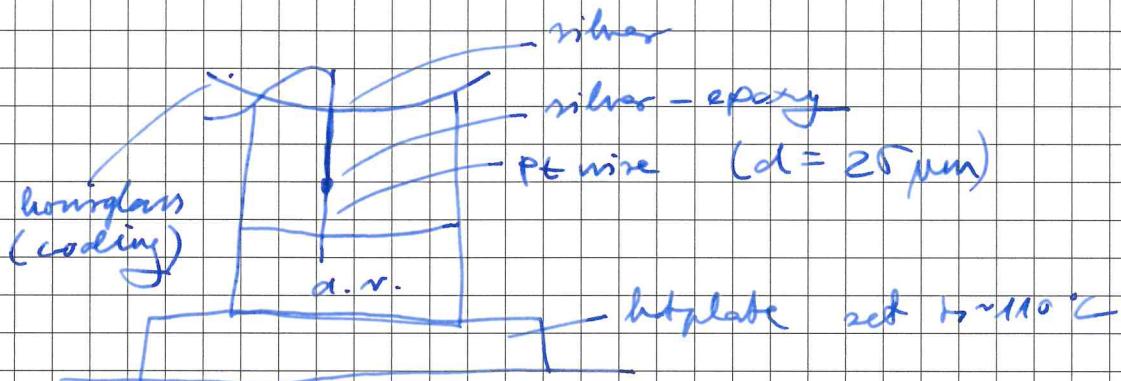
From Page No. \_\_\_\_\_

Aqua regia : 1:3 V mixture of  $\text{HNO}_3$  and  $\text{HCl}$   
 mole ratio  
 $\text{HCl}$        $\text{O}_2^-$   
 $\text{HCl}$        $\text{N}^+ = \text{O}$   
 $\text{HCl}$        $\text{HO}'$

(Mende)  $[\text{HNO}_3] = 6.66 \frac{\text{mol}}{\text{dm}^3}$  (not cc.) this was a very old solution  
 $[\text{HCl}] = 11.65 \frac{\text{mol}}{\text{dm}^3}$ , 321. (cc)

2 ml  $\text{HNO}_3$  sol.  
 3.3 ml  $\text{HCl}$  sol.

$t \approx 100^\circ\text{C}$



etch start : 13:00  
 finish : 13:40

10 μm Pt wire was etched down to ~2.5 μm

To Page No. \_\_\_\_\_

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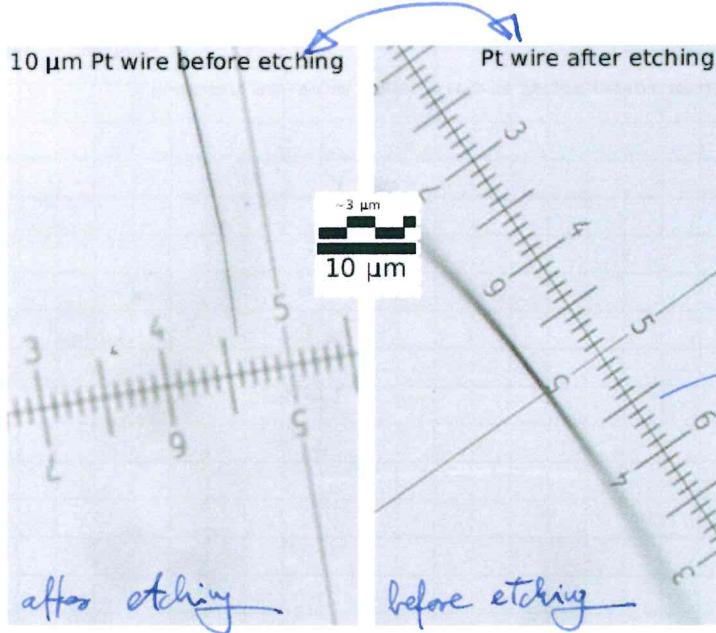
Date

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TITLE \_\_\_\_\_

Book No. \_\_\_\_\_

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To Page No. \_\_\_\_\_

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From Page No. \_\_\_\_\_

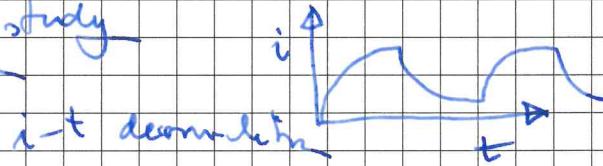
- 1, introduction ↗ what is SECM
- 2, previous work ↗ potentiometry
- 3, SECM Conference Würm
- 4, question: "Can it be done for amperometric SECM?"

5, YES: page 3. ↗ amperometric cell, feedback ...

6, I've started a vigorous study ↗

7, glass sheet ↗

8, Pt wire ↗



↙ deconvolution worked surprisingly well!

9, cells :  $\text{H}_2\text{O}_2$  sink  
 ~~$\text{H}_2\text{O}_2$  source~~  
 it's this one ↗  $\text{O}_2$  sink  
 2 cells ✓

10, spatial deconvolution

PSF

:

11, thank you ↗ photos from PES

To Page No. \_\_\_\_\_

Witnessed and understood by me

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Invented by

Date

160726

Recorded by

TITLE Additional measurements

From Page No. \_\_\_\_\_

- 1, O<sub>2</sub> reduction above glass sheet / bulk
- 2, ~~O<sub>2</sub>~~ change response
- 3, very slow scan (O<sub>2</sub> reduction) for  
spatial deconvolution (almost impossible)  
0.1  $\mu\text{m}/\text{s}$ ?

- with circular Pt electrode 10  $\mu\text{m}$
- electrolyte: PBS + 10 mM glucose (same as for monocytes)

O			
algorithm	speed $\mu\text{m}/\text{s}$	membrane	fast comb
optical and electrochemical images are horizontally mirror of each other	1	12	
	2		
	5		
	10	E2-4	E2-3
	20	E2-5	E2-6
	50	E2-8 & 7	E2-9
	100	E2-11	E2-10

To Page No. \_\_\_\_\_

software

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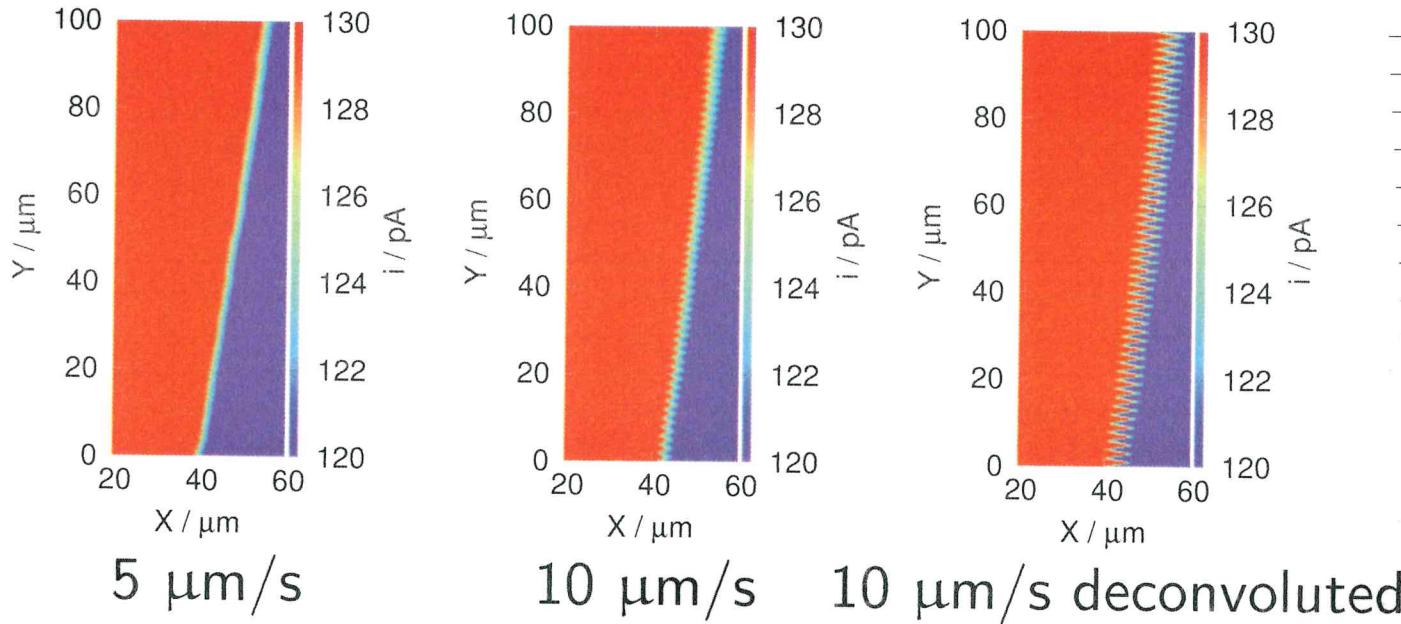
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TIF: 3  
E2-13 is far spatial deconvolution! 53

From Page No. \_\_\_\_\_



Referred to from page 24.



e No. \_\_\_\_\_

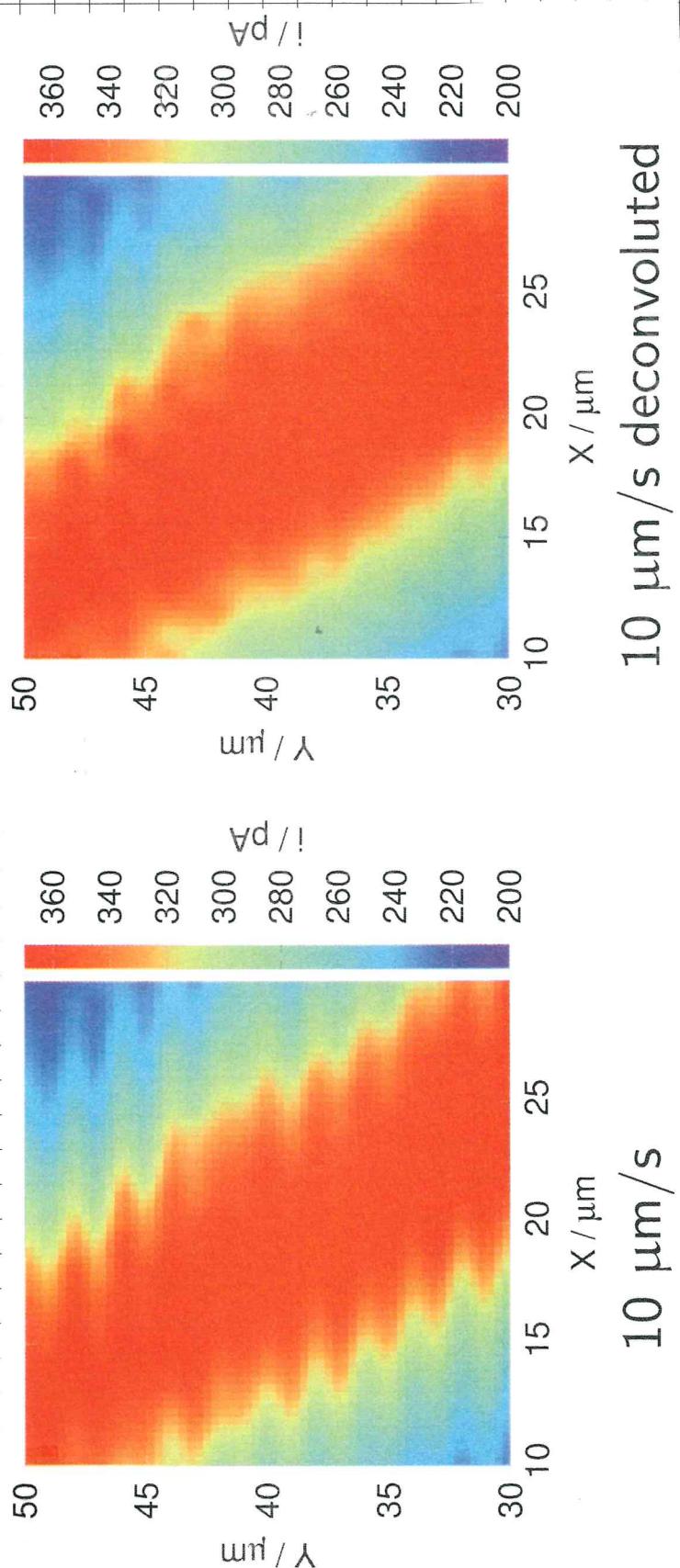
optical image is mirrored  
originally because  
it's taken with the image  
inverses

Project No. \_\_\_\_\_

TITLE \_\_\_\_\_

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Date \_\_\_\_\_

Invented by \_\_\_\_\_

Date \_\_\_\_\_

Recorded by \_\_\_\_\_

TITLE Deconvolution of already slow image

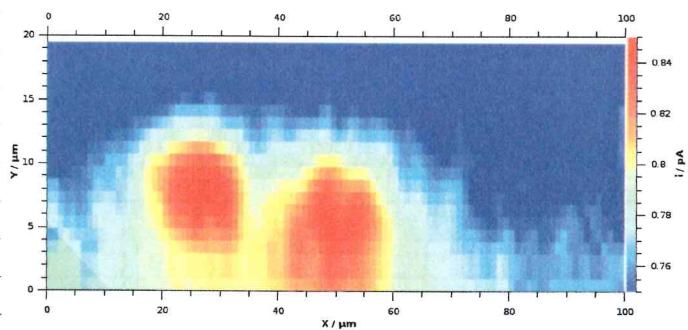
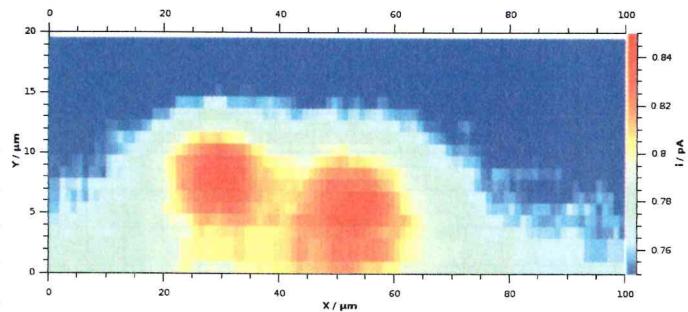
From Page No. \_\_\_\_\_

Targets: 2 monocytes stimulated with TPA.



extracellular  $H_2O_2$   
conc. increases.

scan rate:  $2 \mu\text{m}/\text{s}$



From: 2014. april 1.

There isn't much improvement. The image was already pretty good.

To Page No. \_\_\_\_\_

Witnessed and understood by me

Date

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Recorded by

From Page No. \_\_\_\_\_

```

#!/usr/bin/enc python

'''

Deconvolution of distorted SECM images.
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along with this program; if not, write to the Free Software
Foundation, Inc., 51 Franklin Street, Fifth Floor, Boston,
MA 02110-1301, USA.

Here is a first attempt at porting the deconvolution algorithm
from FORTRAN to python. The gaussian filter is not yet implemented
in the program. Right now I do it with the plotting software (gnuplot),
but it would be better if the python program did it. Also, I haven't
done the command line argument interpreter yet, so the file name must
be changed in the code every time. A GUI would be nice, and a live plot
of the convoluted and deconvoluted image. For that, the XYZ data needs
to be converted to a matrix.

'''

import numpy as np
import subprocess

conv_img = np.loadtxt("9_41_meandered.txt")
deconv_img = np.copy(conv_img)
e0 = np.float32(conv_img[0][2])
for n in range(0, conv_img.shape[0]):
    deconv_img[n][2] = np.float32((conv_img[n][2]-e0*0.68)/(1-0.68))
    e0 = np.float32(conv_img[n][2])

np.savetxt("9_41_meandered_deconvoluted.txt", deconv_img, delimiter=" ")

#proc = subprocess.Popen(['gnuplot', '-p'],
#                       shell=True,
#                       stdin=subprocess.PIPE,
#                       )
#proc.stdin.write('set xrange [0:10]; set yrange [-2:2]\n')
#proc.stdin.write('plot sin(x)\n')
#proc.stdin.write('quit') #close the gnuplot window

```

The deconvolution algorithm in Python.  
I've written it on 2018.07.02.

To Page No. \_\_\_\_\_

Witnessed and understood by me

Date

Invented by

Date

Recorded by