TOWARDS Electronic Detection of Genomic-length DNA with no labels

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Outline:

1) The importance of single cell large scale genomic length mapping

2) Troubles with present single cell technologies

3) The advantages of nanochannels for large scale genomic length mapping

4) Beyond optics: electronic detection?

5) Quo vadis?

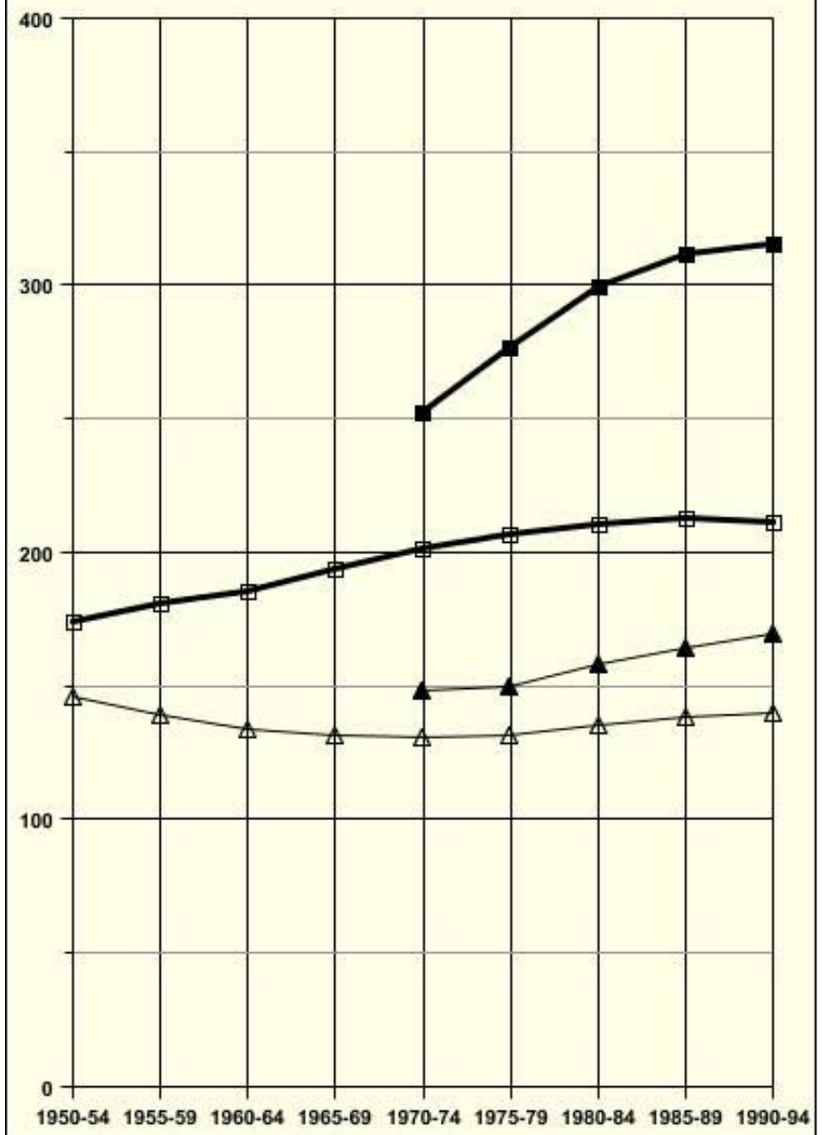
1) The importance of single cell large scale genomic length mapping

"All happy families are the same, all unhappy families are unhappy in different ways"

-Tolstoy

5-year Cancer Mortality Rates per 100,000 person-years,
Age-adjusted 1970 US Population
All Cancers, 1950 to 1994, All Ages

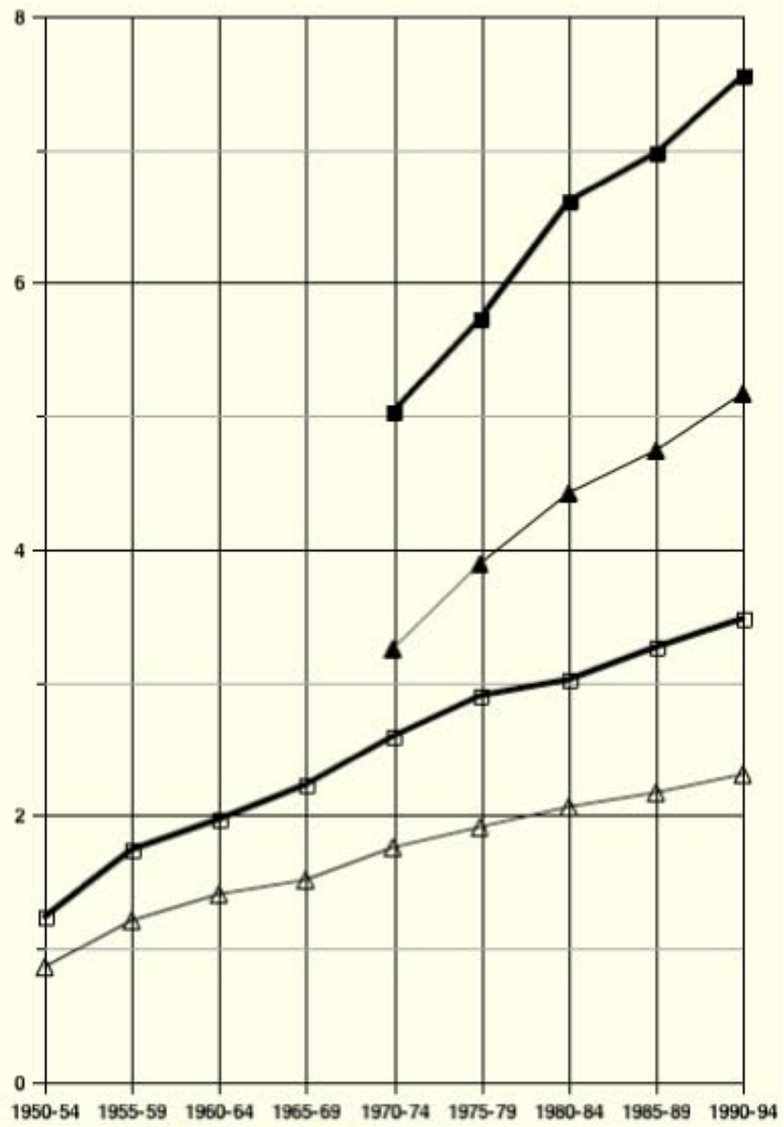
(A)



Source: Cancer Mortality Maps & Graphs Web Site,
a service of the National Cancer Institute
http://cancer.gov/atlasplus/

5-year Cancer Mortality Rates per 100,000 person-years,
Age-adjusted 1970 US Population
Multiple myeloma, 1950 to 1994, All Ages

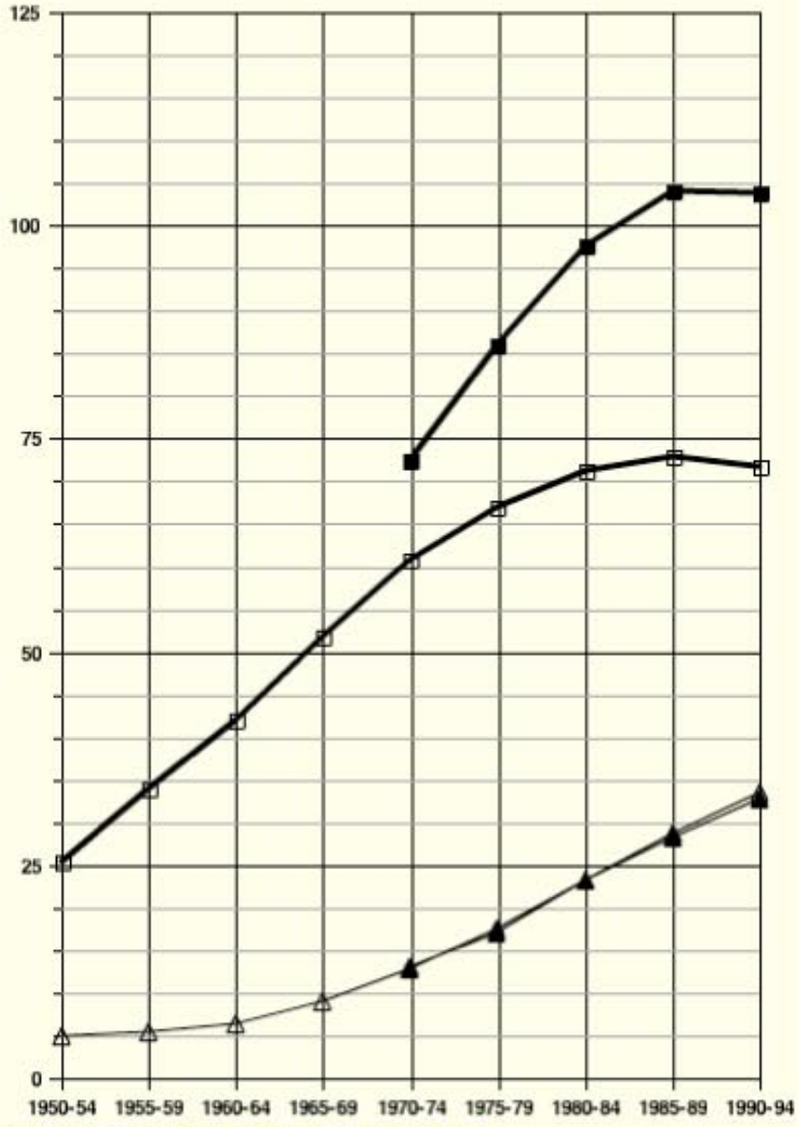
(B)



Source: Cancer Mortality Maps & Graphs Web Site,
a service of the National Cancer Institute
http://cancer.gov/atlasplus/

5-year Cancer Mortality Rates per 100,000 person-years,
Age-adjusted 1970 US Population
Lung, trachea, bronchus, pleura, 1950 to 1994, All Ages

(C)



Source: Cancer Mortality Maps & Graphs Web Site,
a service of the National Cancer Institute
http://cancer.gov/atlasplus/

WORLD WAR ON CANCER MAKES NOTABLE GAINS

Practical Employment of All Available Information and Promotion of Research Regarding the Disease Have Resulted in More Cures and Preventions

PHYSICIANS and research laboratories in many countries are engaged in the war against cancer. The progress of this war is described in the following article. Dr. Soper was for six years director of a national society against cancer and in this capacity visited almost every centre in America and Europe where cancer research is carried on. His latest investigations were made in Europe during the past Summer.

By GEORGE A. SOPER, Ph. D.

NOTABLE progress is being made in the war against cancer. The strength and disposition of the enemy's forces are coming to be better understood. Equipment and methods are being developed which make it possible more and more successfully to combat the foe. Some of the strongest positions are being taken.

The terror with which cancer has been regarded for hundreds of years is no longer warranted. Cancer is undoubtedly one of the world's greatest plagues, but it is by no means the invariably fatal malady which it was once believed to be. Many more cases are being cured and many more prevented than formerly. This is being done partly through the greater cooperation of the patients, who are learning the necessity for early and skillful attention, and partly through the more general employment of methods based on a better understanding of the cancer process.

As time passes it becomes more and more clear that what is called cancer is not a single disease with a definite and invariable train of symptoms, but rather an unwholesome biological condition which gets started in some way or other and cannot always be checked. To effect a cure, the part or parts of the body involved must be so treated as to destroy or remove every particle of the cancerous tissue. The full extent of the growth is invisible, but its probable location must be known to the physician if the patient is to be permanently cured. The successful treatment of a difficult case of cancer is a very scientific accomplishment.

cific kind of parasite. The virus theory of Gye, which aroused so much popular and scientific interest when announced through The Lancet a few years ago, has been the subject of a great deal of research work in all parts of the civilized world, without, however, being confirmed.

The resistance which the normal body ordinarily offers to the cancer process is receiving a good deal of attention from qualified research workers, and the fundamental reasons why it becomes lowered or raised are being carefully investigated.

Second, a great deal of research is being carried on into the immediate, as distinguished from the fundamental, causes of cancer. It has become plain that while susceptibility plays an important part, it is only one of two great factors, both of which are probably concerned in the production of every cancer. The other is some form of local irritation, usually acting over a long interval of time. It is the combination of these two, rather than either one of them acting alone, which produces a cancer. If either is present to a marked degree, the other need not be so.

One thing is obvious: All forms of irritation are not equally dangerous. New light is constantly being shed on the forms which are most likely to lead to cancer and, consequently, especially to be avoided. Certain tars, oils and aniline dyes have been implicated, for example. Practical information has thus been obtained as to the necessity of precautions to be taken by operatives in various industries. Attempts have been made to produce such a substance as may be assumed to occur naturally in living tissues before a cancer is formed. It is probable that if this can be obtained a means of counteracting the disease can be found.

Another line of research with a practical bearing has been the study of the relationship between physical injury and the production of cancer, for it is popularly supposed that a cancer sometimes follows a blow or wound. The result of this inquiry leads to the opinion that the connection, if any, is much less direct than has been commonly supposed. Attempts made to produce cancer of the lung in animals by methods resembling the smoking of cigarettes have failed. These are examples of recent research work into the immediate or exciting causes of cancer.

Third, the development of methods and facilities for diagnosis and treatment. In this direction lies the greatest advance which has been made in recent years against cancer. Through improvements in technique, and, still more, through the intelligent recording and study of what has been accomplished in groups of similar cases treated in the same way, the percentage of cures has steadily risen. This is especially true of radium and X-ray treatment, when employed alone or in combination with surgery. The Radiumhemmet at Stockholm and the Radium Institute of the University of Paris are universally regarded as leaders in this direction.

Irradiation has steadily advanced as one of the best ways to treat cancer, but it is only a tool and, like all tools, the results which are to be obtained with it depend upon the knowledge and skill of the one who uses it. Today irradiation stands on an equal footing with surgery and is actually superior to it in dealing with cancer in certain parts of the body.

Medical Treatment.

The medical cure of cancer remains where it was a year ago, when the discussion of this subject by a special section of the International Cancer Conference at London furnished the only disappointment of the meeting. Up to the present no medicine or drug or serum or vaccine suitable for general use and capable of curing cancer has been recognized by the medical profession. Only surgery or X-rays or radium in their established forms and combinations should be depended on.

The methods of treating cancer by irradiation which are universally regarded as best have been brought to their present state by a comparatively few individuals and institutions. Last year the League of Nations asked some of the foremost authorities to compare their methods and draw up a report giving detailed instructions to cover the procedures which they considered best. This has been done, and a report was issued last June which is of great value.

The Cancer Commission of the League of Nations is composed of eminent scientists from England, France, Germany, Switzerland, Italy, Holland and Japan. It attracted much notice two years ago by publishing a statistical report which showed that the cancer mortality in certain parts of the body was quite different in different countries without any reason for it being apparent.

On July 24 last the House of Commons voted about $500,000 to equal the same amount raised by popular subscription with the assistance of the Thanksgiving Fund for the King's recovery, the intention being to purchase twenty grams of radium to be used chiefly for the treatment of cancer. The quantity previously in the country has been estimated at about twenty-six grams. The popular subscription was raised in record time. On April 29 The London Times published a strong appeal for contributions and within ten days about $900,000 had been given.

A Radium Trust has been established, with a royal charter, to hold this money and be responsible for the ownership of the radium, and a commission has been set up for its care and utilization. It is intended that portions of the radium shall be allocated, under appropriate regulations, to institutions which are qualified to use it, but that the actual ownership and ultimate control shall always be vested in Parliament.

How to make the best skill and ability available to all who so sorely need it is the greatest practical problem which is connected with the war against cancer at the present time. Apparently the solution does not lie in making the diagnosis and treatment of cancer a specialty in medicine, as some have proposed. The subject is too complicated and calls for exceptional knowledge and skill in too many directions for that.

A plan which is being followed in some hospitals with good results is the formation of groups, each member of which is expert in some particular branch of the work to be done. A group should contain a pathologist, a general surgeon, a radiotherapist and an internist. If possible there should be specialists in the various branches of surgery, as gynecology, laryngology, &c.

There is need, also, of special equipment. Not only must there be provision for surgery but for X-ray photographs, the therapeutic applications of X-rays and radium, and the keeping of suitable records. In other words, what is required is a cancer clinic or a cancer hospital. We have some already, but there are not nearly enough. It would be a good thing if every general hospital of 200 beds or more had a cancer clinic.

It must not be supposed from what has been said that every case of cancer requires the combined attention of a whole group of experts. There are many cases numerically, although they form a small proportion of all, which occur on the surface, can readily be recognized and can be successfully treated by every surgeon or radiotherapist. It is the obscure and insidious cases, and particularly those which involve internal structures, which call for expert treatment. These happen to be the ones which most often occur.

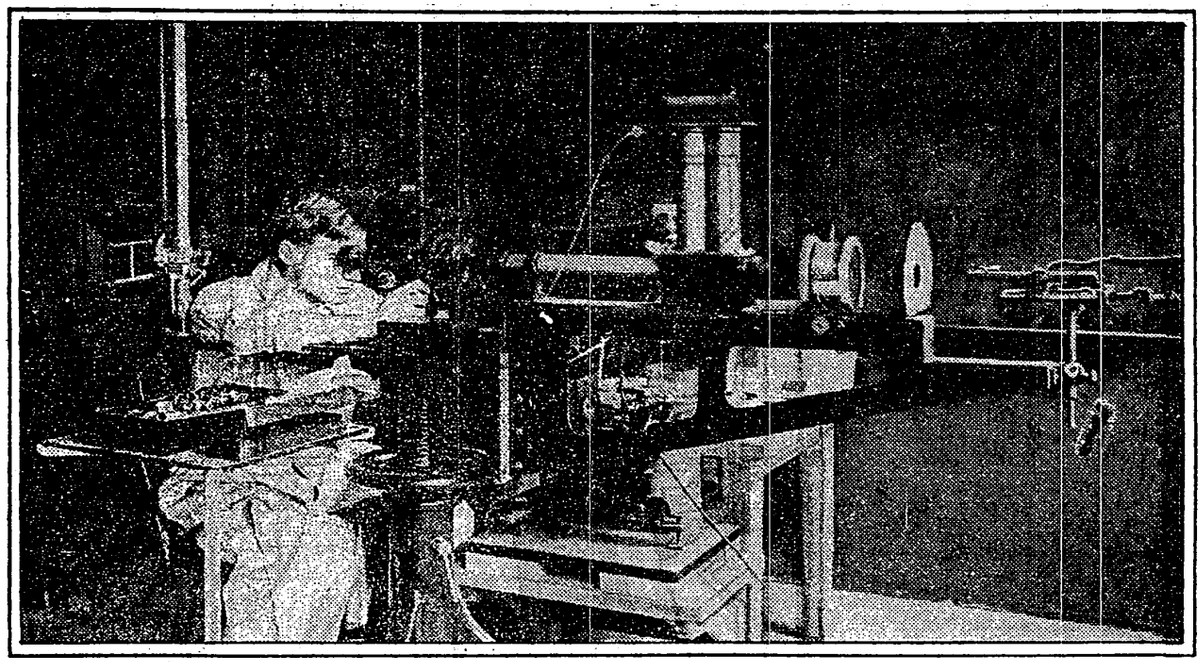
Some Organized Efforts.

The laboratory, clinic and hospital facilities which are now available for cancer patients are rarely adequate in any city. Let us glance for a moment at the efforts which are being made to supply this deficiency in the various parts of the world.

In France anti-cancer centres have been established in all parts of the country, and they now number fourteen. The law under which they function provides that each centre shall contain facilities for treating the sick, for conducting research, and for the instruction of medical students. They are all under the control of the government and receive a part of their support from it. Following this example, a number of other countries have also established centres.

In England there are special cancer hospitals, and wings and wards of many general hospitals are devoted

APPARATUS USED IN CANCER RESEARCH WORK



With the Photo Micrograph It Is Possible to Make Highly Magnified Pictures of the Minute Cellular Structures of the Body and Thus Record Abnormal Changes.

My sister, Linda, died from ovarian cancer. The progression was typical: surgery, chemo, remission followed by relapse after 2 years, which was fatal. Same old story.

What happened to the War on cancer? Where is the Victory?

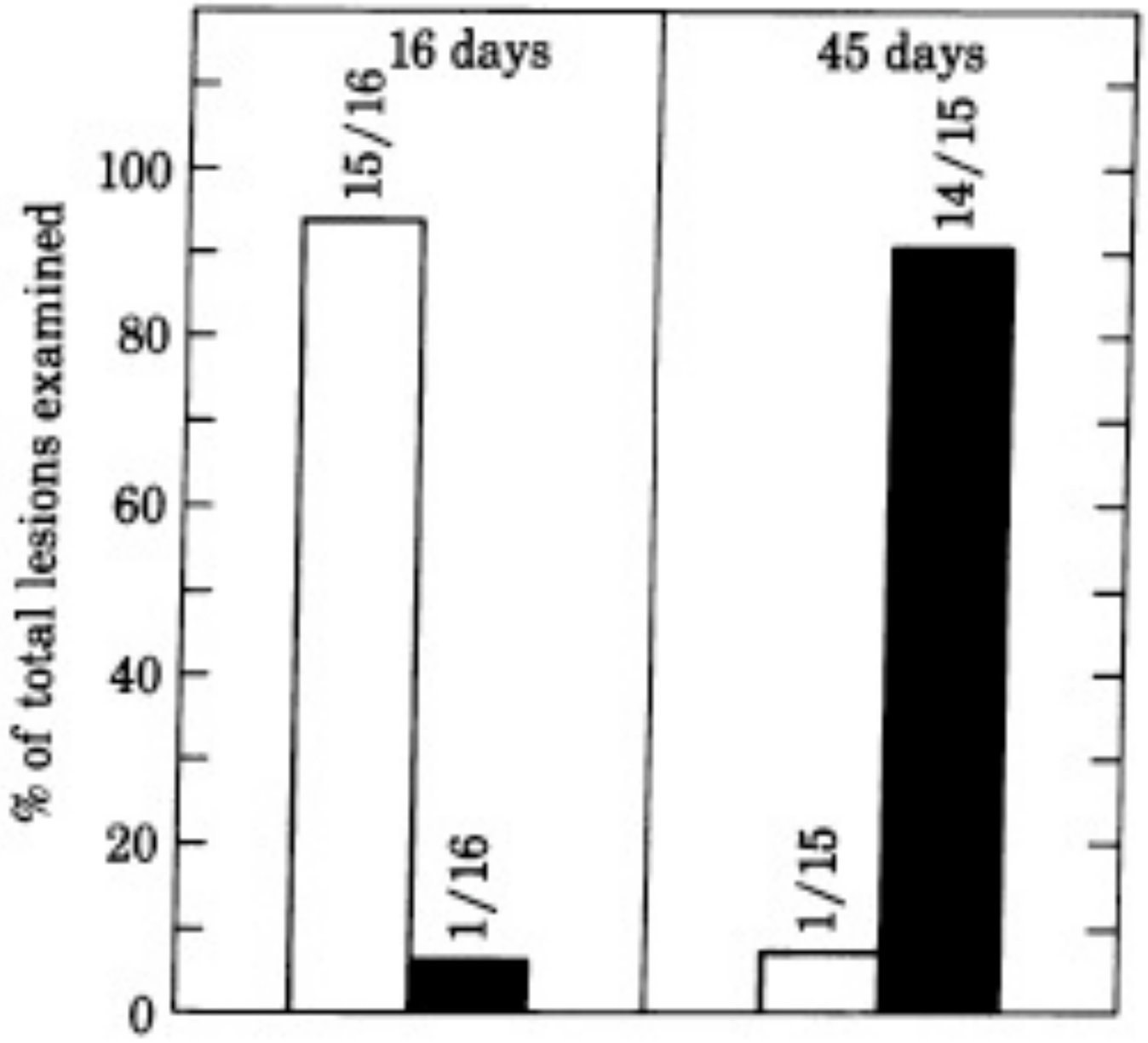
Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 6574–6578, November 1982
Cell Biology

Evolution of tumor cell heterogeneity during progressive growth of individual lung metastases

(cancer/phenotypic stability)

GEORGE POSTE*†, JAMES TZENG†, JOHN DOLL*, RUSSELL GREIG*, DAVID RIEMAN*, AND IRVING ZEIDMAN†

ABSTRACT The metastatic properties of tumor cell clones isolated from individual lesions of B16 melanoma metastatic to lung have been examined at different stages in the evolution of metastasis. Clonal analysis of metastatic lesions produced by B16 melanoma populations containing clones with identifiable, stable drug-resistance markers revealed that the majority (>80%) of experimental metastases produced by intravenous injection of tumor cells are of unicellular origin. During the early stages of their growth (<25 days after initial tumor cell arrest), the majority of metastatic lesions contain cells with indistinguishable metastatic phenotypes (intralesional clonal homogeneity) although different clonally homogeneous lesions from the same host contain tumor cells with different metastatic phenotypes (interlesional clonal heterogeneity). Progressive growth of metastatic lesions is accompanied by emergence, within originally clonally homogeneous lesions, of variant tumor cells with altered metastatic properties (intralesional clonal heterogeneity). By 40–45 days after initial arrest of injected tumor cells in the lung, 90% of the metastatic lesions are populated by cells with heterogeneous metastatic phenotypes.


16 days
45 days
15/16
14/15
1/16
1/15
% of total lesions examined
100
80
60
40
20
0

The genome in cancer cells undergoes rapid evolution under conditions of high stress.

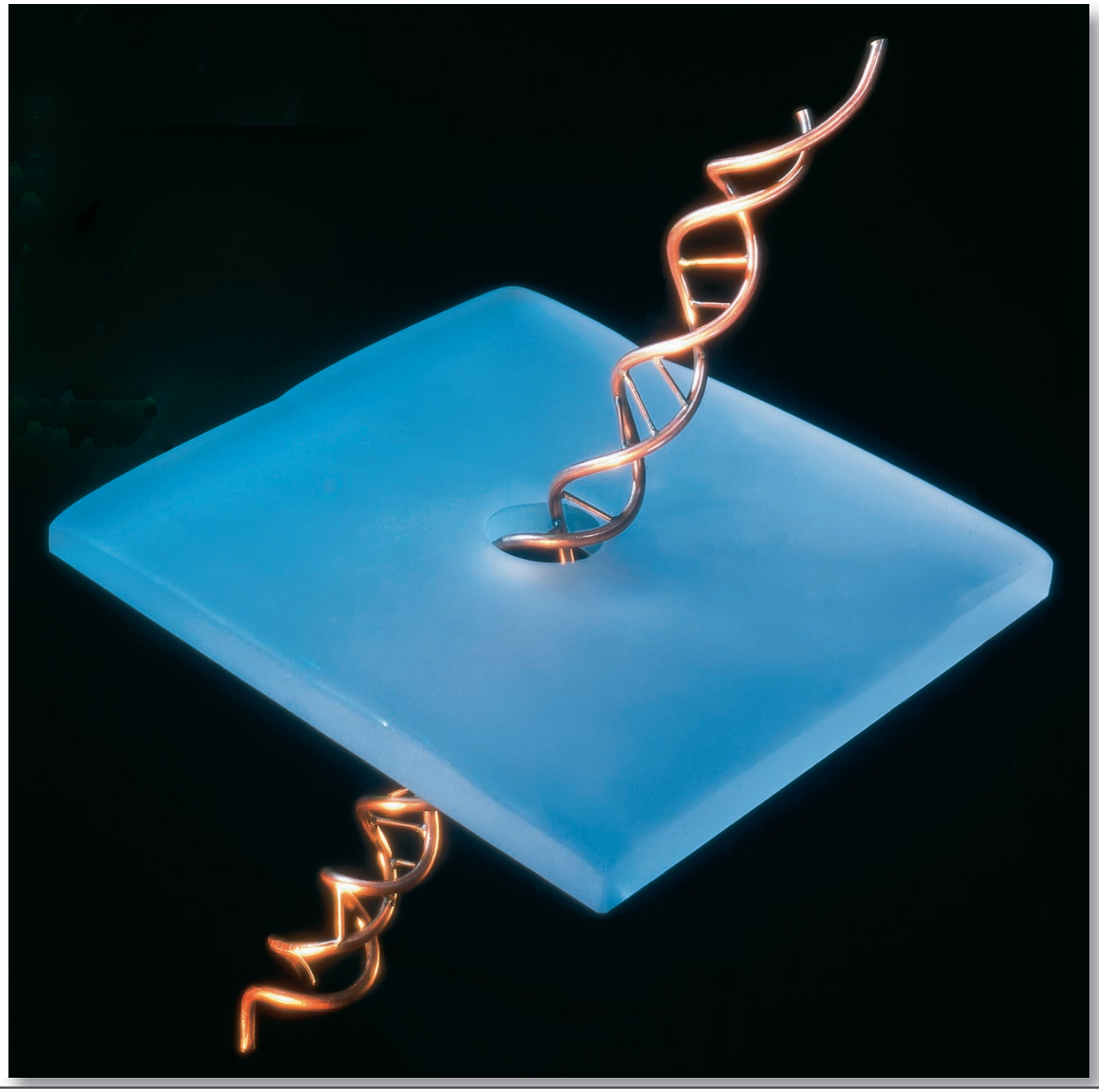
Sequencing is hopelessly specific for mapping these rapid and large scale genomic rearrangements, and present techniques cannot map single cells rapidly and with high spatial resolution.

2) Troubles with present technologies

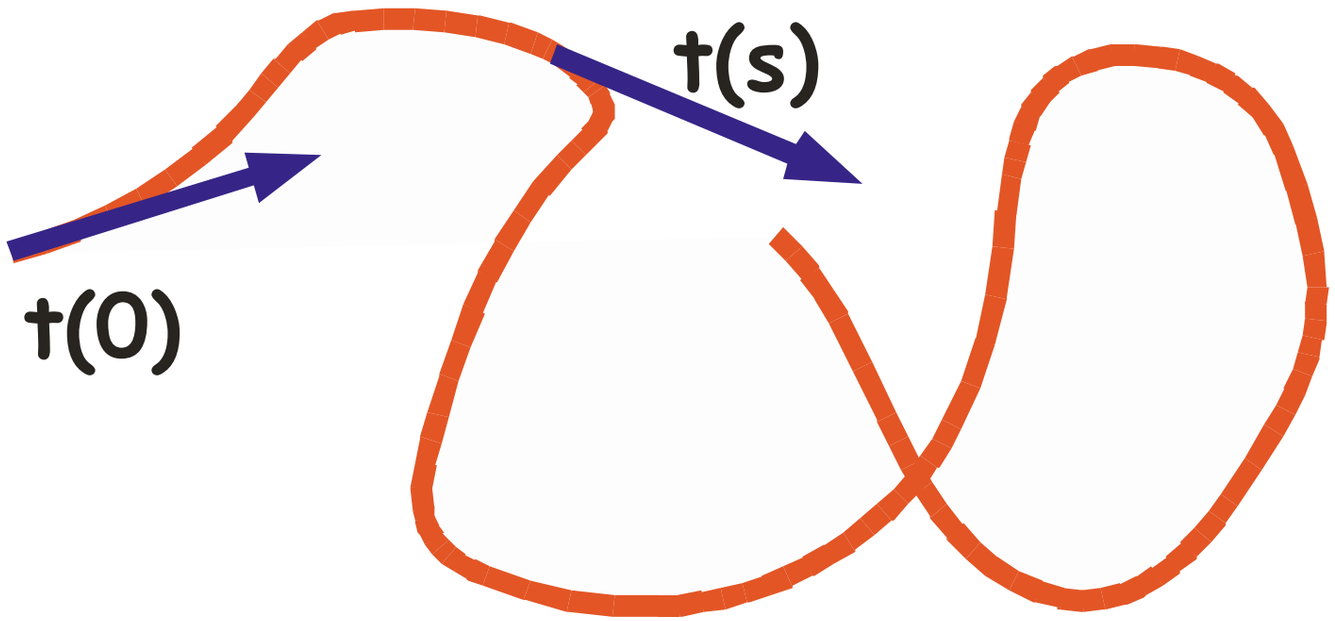
NANOPORES

The art of sucking spaghetti

Biomolecules are notorious for their unpredictable flexibility. Some of the smallest nanopores ever created are being used to manipulate individual DNA molecules, with far-from simple results.

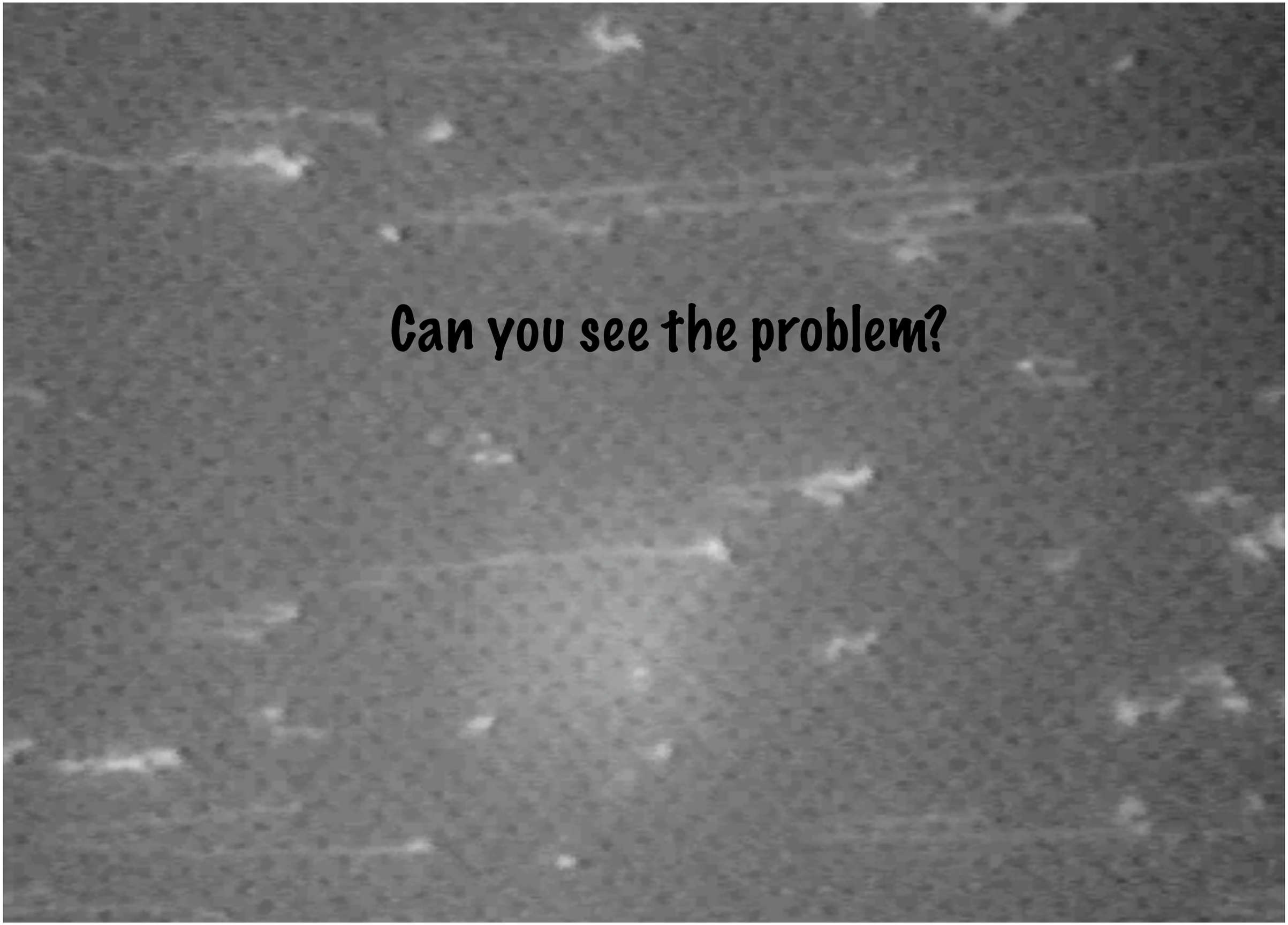


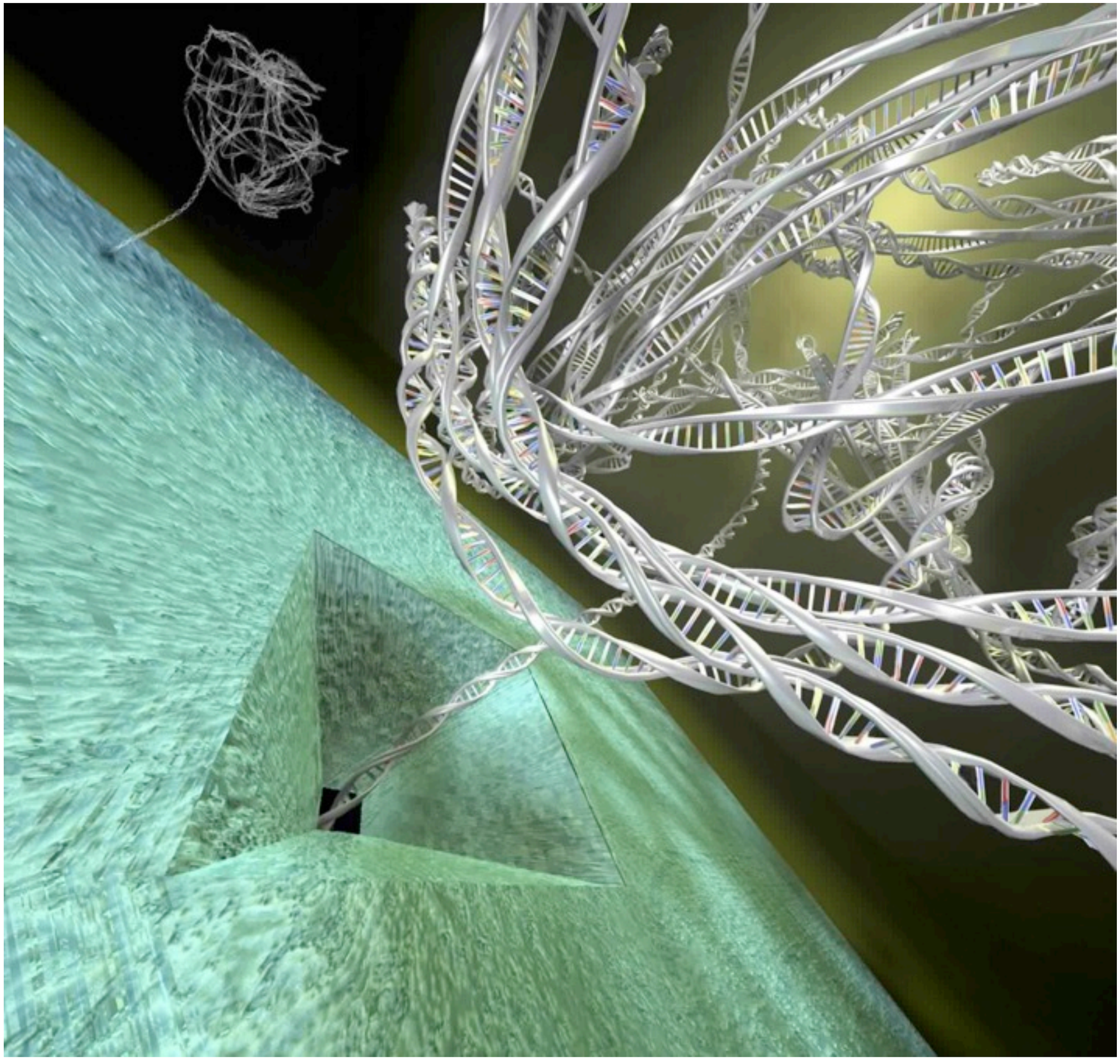
A very important concept here: the persistence length "p" of a flexible polymer. Basically, it is a measure of how far you move along an arc before thermal energy bends the polymer randomly.



movie:wayne

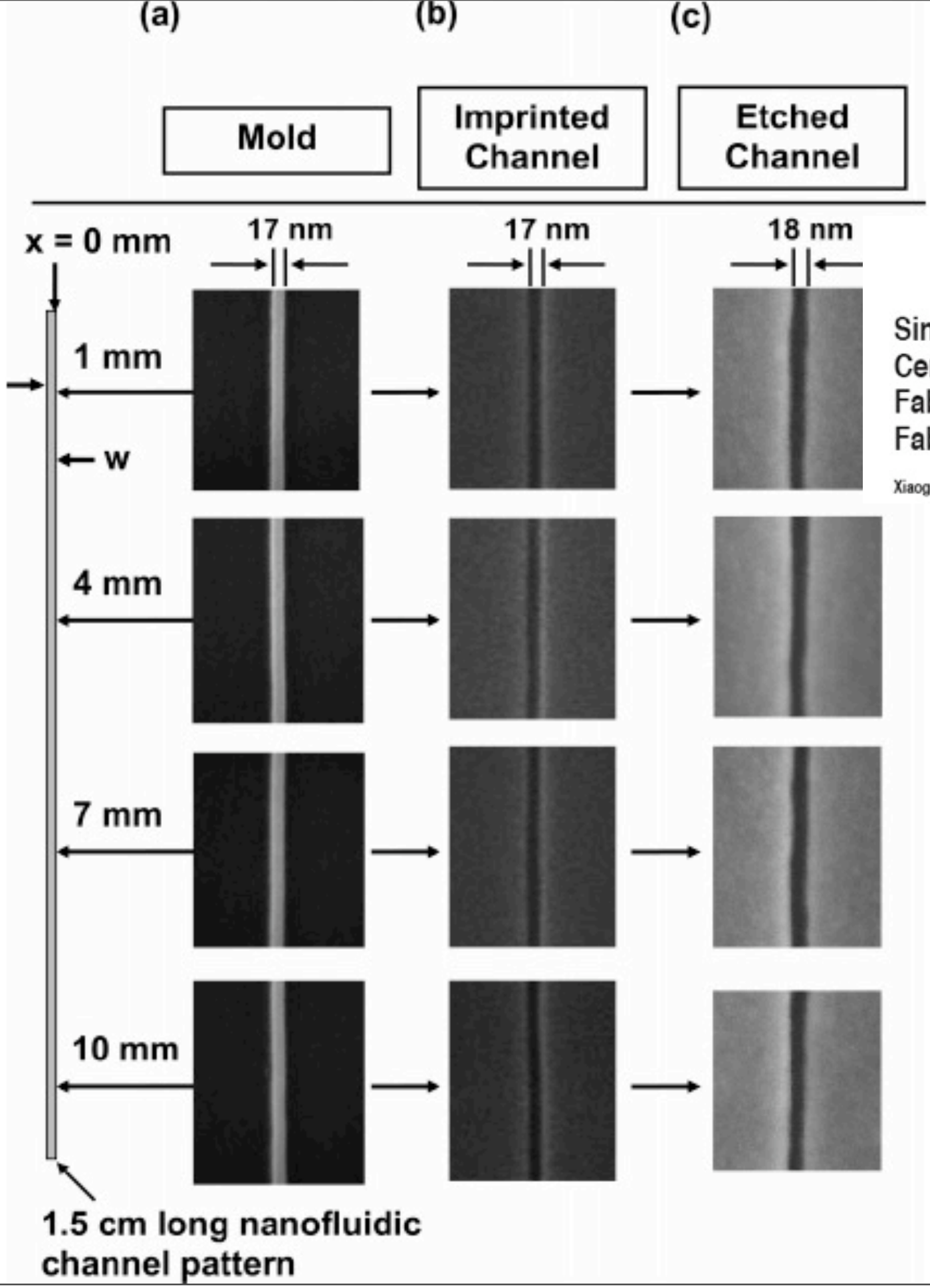
<t(0) t(s)> = exp(-s/p) p = 600 Angstroms = 200 bp (double-strand DNA)


Can you see the problem?



Dekker et al. Notice the magic.

3) The advantages of nanochannels for large scale genomic length mapping



NANO LETTERS
2007
Vol. 7, No. 12
3774−3780

Single Sub-20 nm Wide, Centimeter-Long Nanofluidic Channel Fabricated by Novel Nanoimprint Mold Fabrication and Direct Imprinting

Xiaogan Liang,† Keith J. Morton,† Robert H. Austin,‡ and Stephen Y. Chou*,†

Here's an interesting polymer problem: what happens when you put a long polymer of persistence length p in a nanochannel? Lot's of surprises.

Suppose the channel is say 200 nm wide, and the polymer has a persistence length of 50 nm. The diameter of the dsDNA molecule is only about 2 nm, so most of the volume of the channel is water, since the diameter of the polymer is much less than the persistence length or the channel dimension.

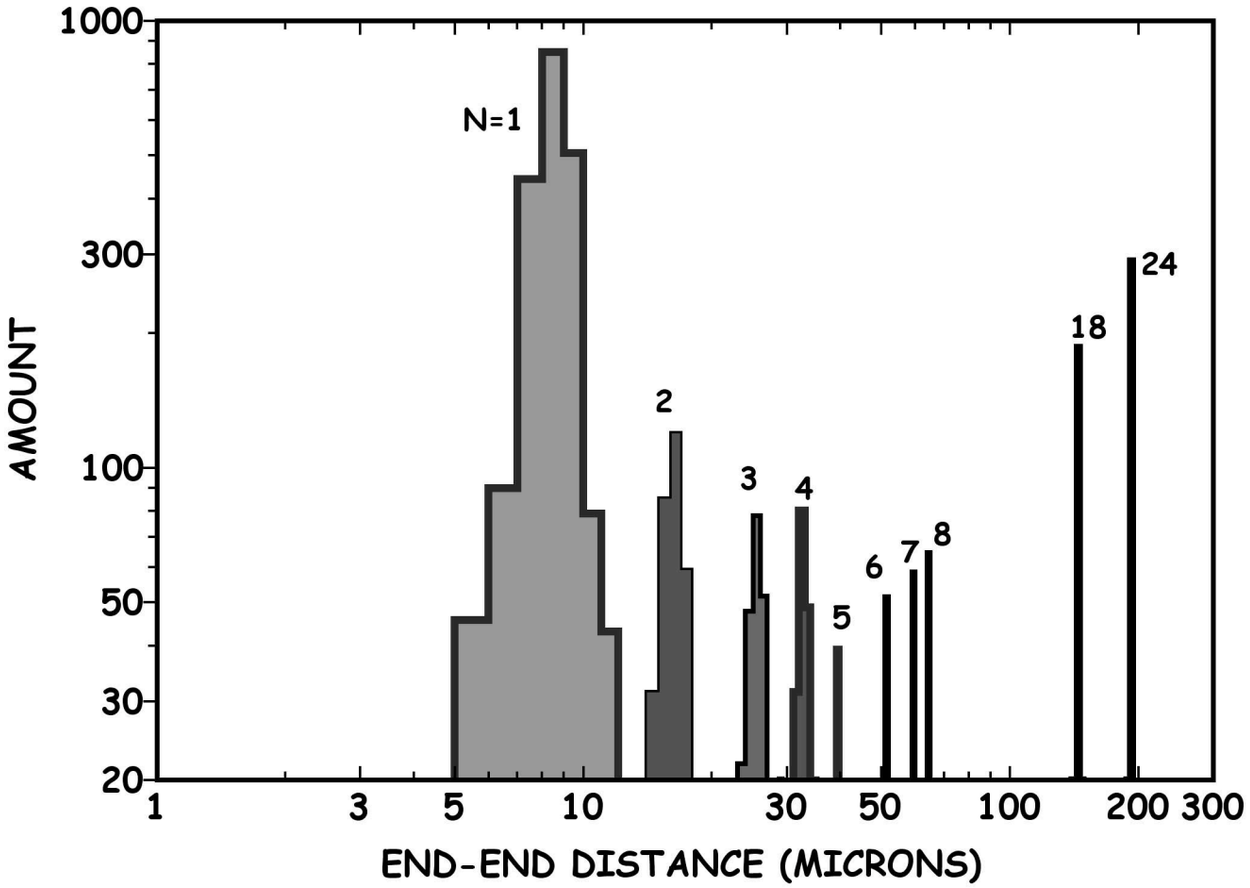
You might think that the self-avoiding random walk would be an unnecessary complication.

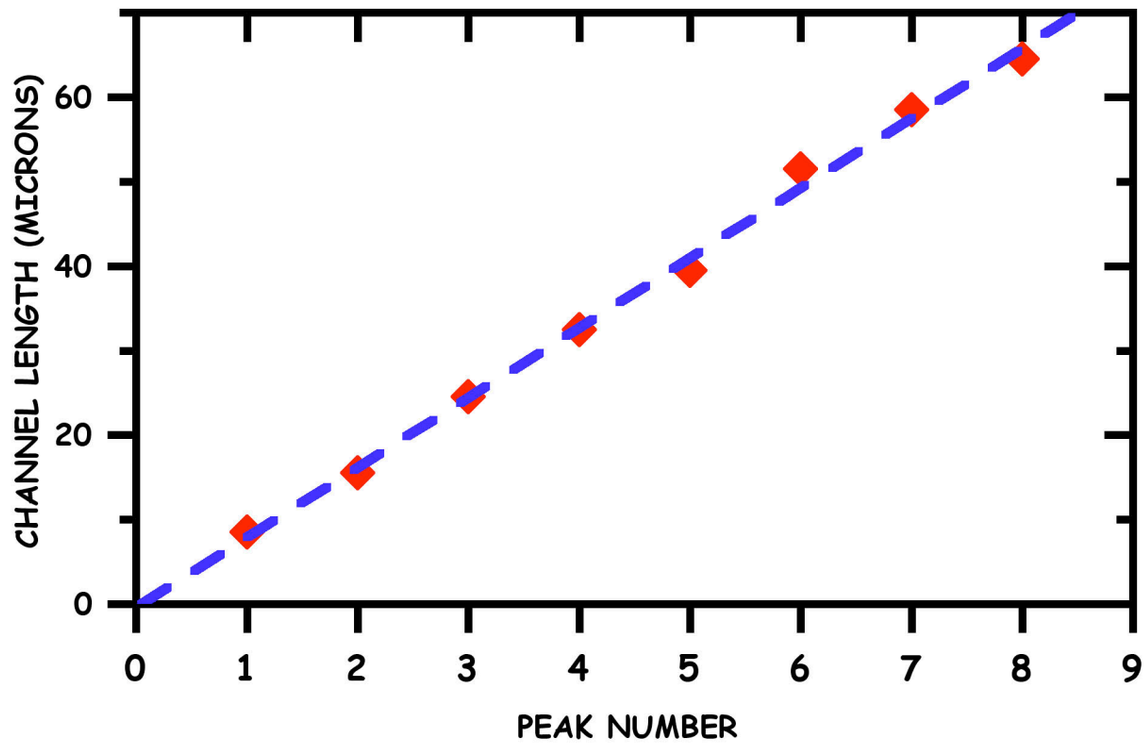
If the polymer has contour length L, the end-end length in a tube of diameter D for a polymer of width w is:

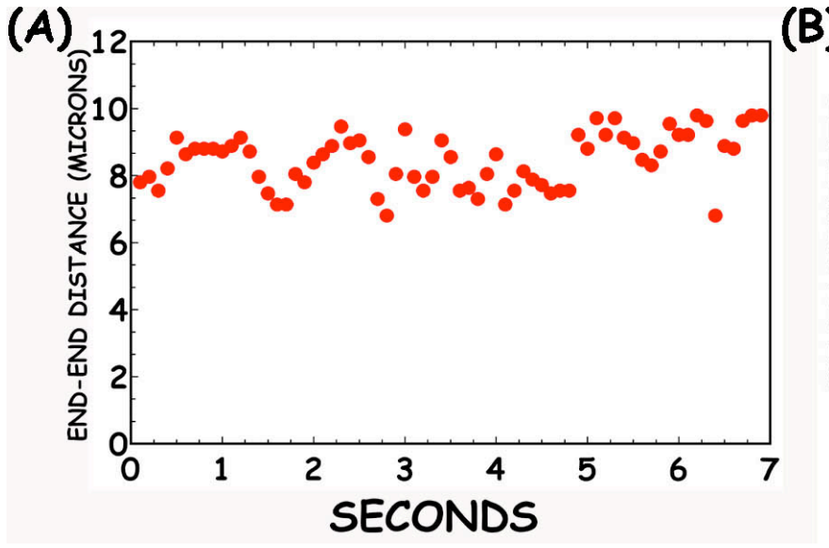
$$L_z = L\frac{(pw)^{1/3}}{D^{2/3}}$$

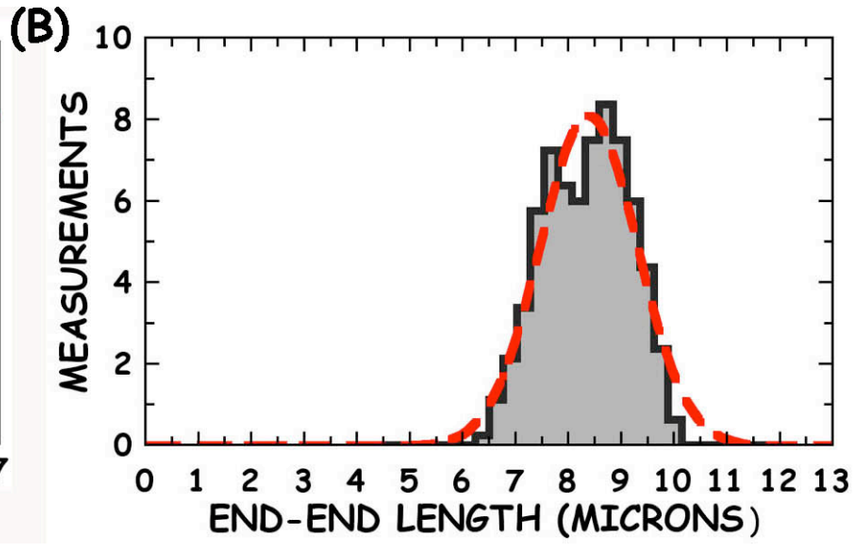
The entropic spring constant k of this confined polymer is:

$$k \simeq \frac{15}{4}\frac{k_B T}{L}\left[\frac{1}{pwD}\right]^{1/3}$$

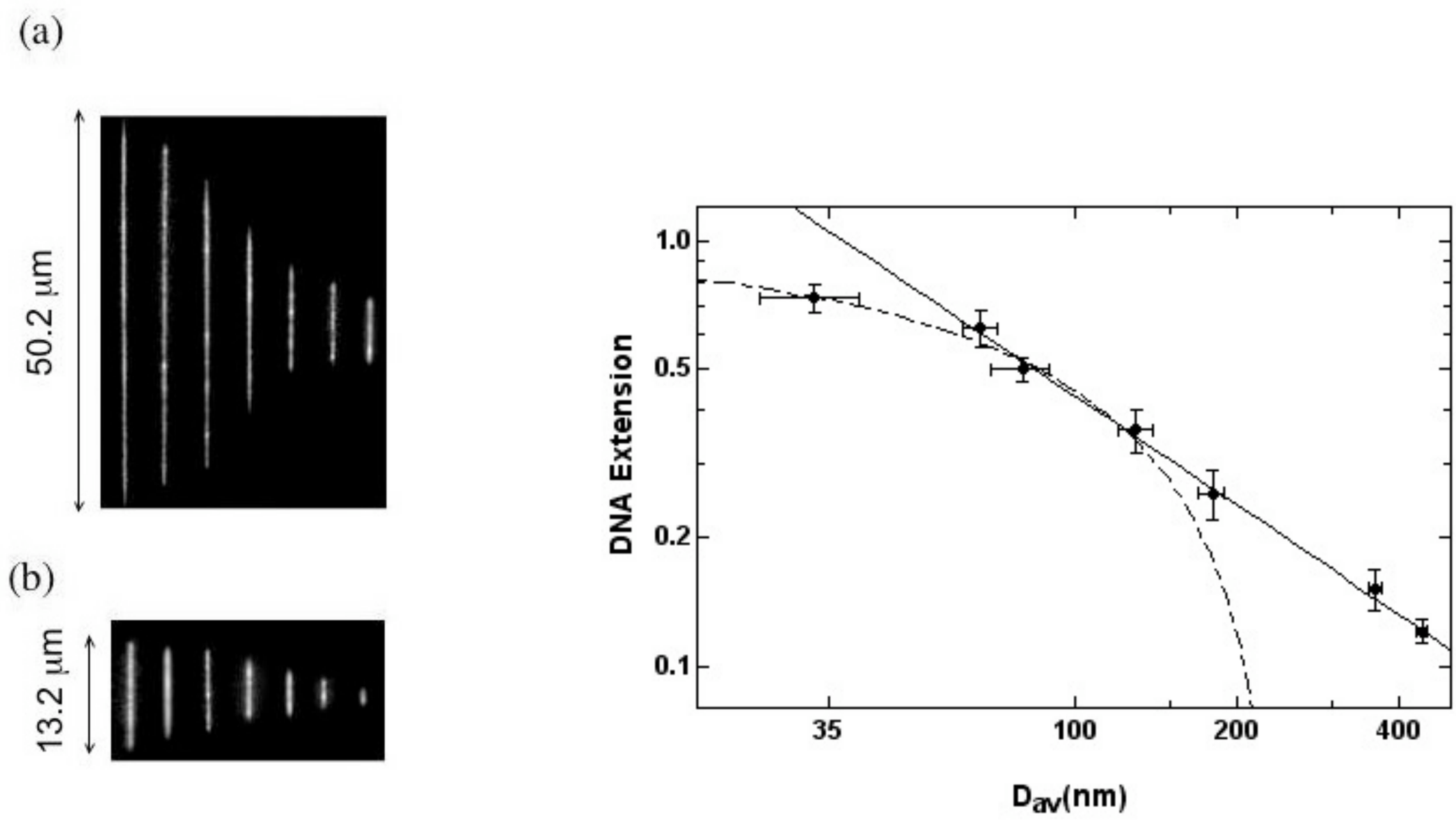

1000
300
100
50
30
20
AMOUNT
N=1
2
3
4
5
6
7
8
18
24
1
3
5
10
30
50
100
200 300
END-END DISTANCE (MICRONS)


CHANNEL LENGTH (MICRONS)
0
20
40
60
0 1 2 3 4 5 6 7 8 9
PEAK NUMBER


(A)
END-END DISTANCE (MICRONS)
0
2
4
6
8
10
12
0 1 2 3 4 5 6 7
SECONDS


(B)
MEASUREMENTS
0
2
4
6
8
10
0 1 2 3 4 5 6 7 8 9 10 11 12 13
END-END LENGTH (MICRONS)

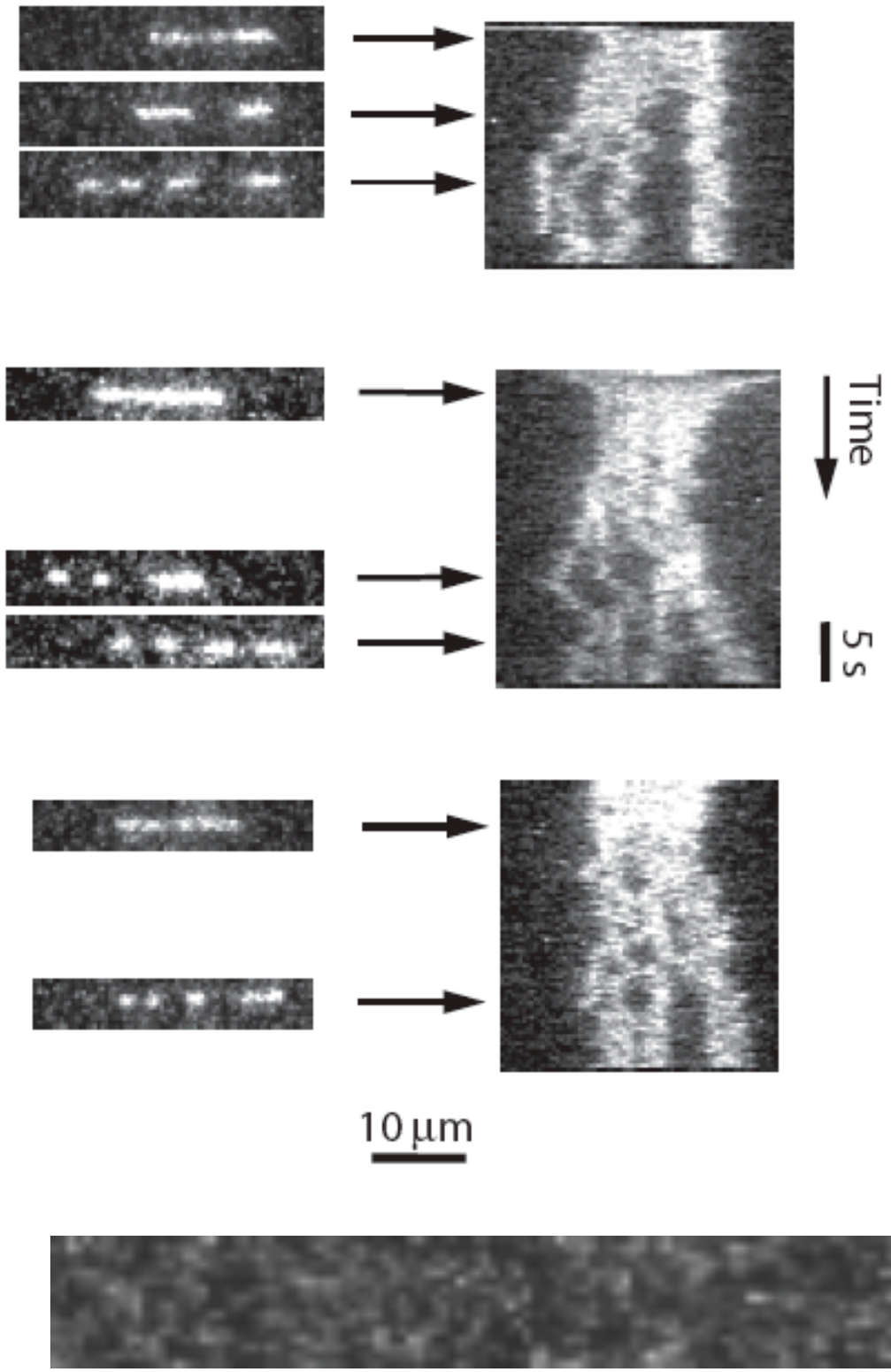
$$L_z = Lcos(\theta) = L\left[1 - A\left(\frac{D}{P}\right)^{2/3}\right].$$

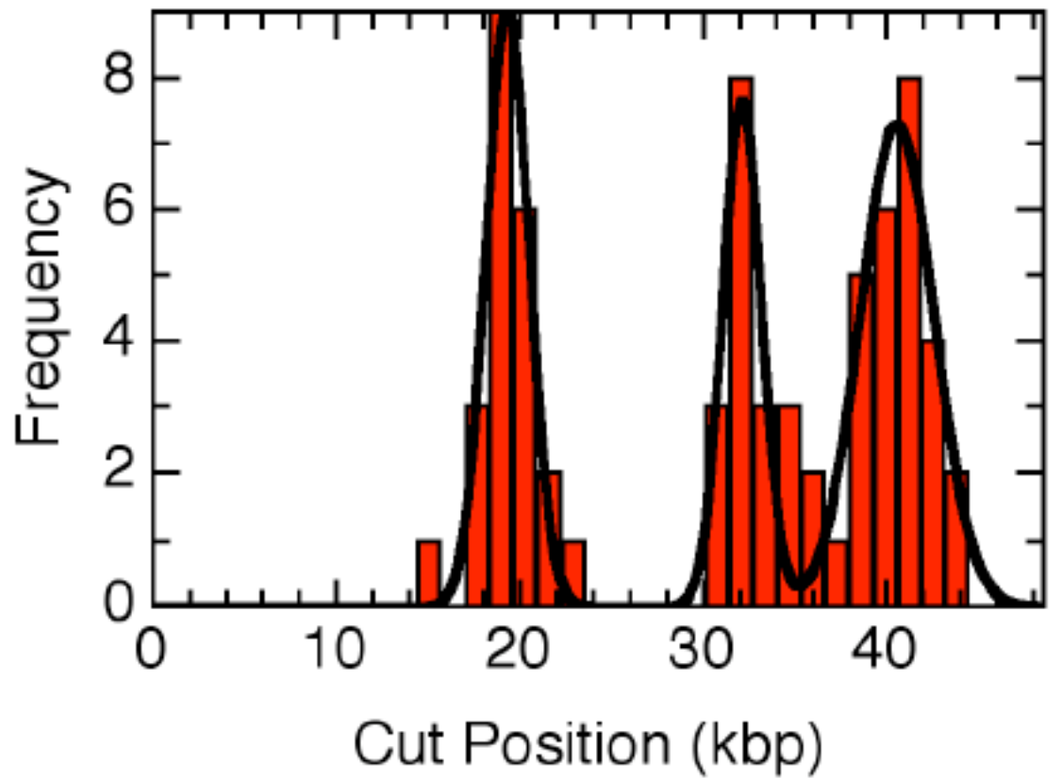


Restriction mapping of DNA with endonucleases is a central method of modern molecular biology. It is based on the measurement of fragment lengths after digestion, while possibly maintaining the respective order.

Robert Riehn decided that perhaps we could bring restriction enzymes into these nanochannels and cut genomic length DNA molecules at precise sites. Since we would observe the cutting directly, there would be no scrambling of the order of the cut sites, and so we could do a direct physical map of a DNA of genomic length.

This has been a hard road to go down!


Time
5 s
10 µm



Sequence	19.4	31.6	39.9
Histogram	19.3 ± 1.2	32.1 ± 1.0	40.6 ± 2.0
Weighted Average	19.9 ± 1.3	32.8 ± 1.3	40.7 ± 1.7

4) Beyond optics: electronic detection

Central idea: combine nanochannel elongation with "electronic" detection of the charged DNA molecule, either by nanoelectrodes directly put into nanochannels or via nanpores serially connected with nanochannel.

Some warnings:

1) Debye length (shielding of charged phosphate groups by saline counter-ions is quite small (nm or less), creates charge double-layer.

$$\frac{1}{\kappa} = \sqrt{\frac{\epsilon\epsilon_o k_B T}{e^2 \sum_i c_i^\infty z_i^2}}$$

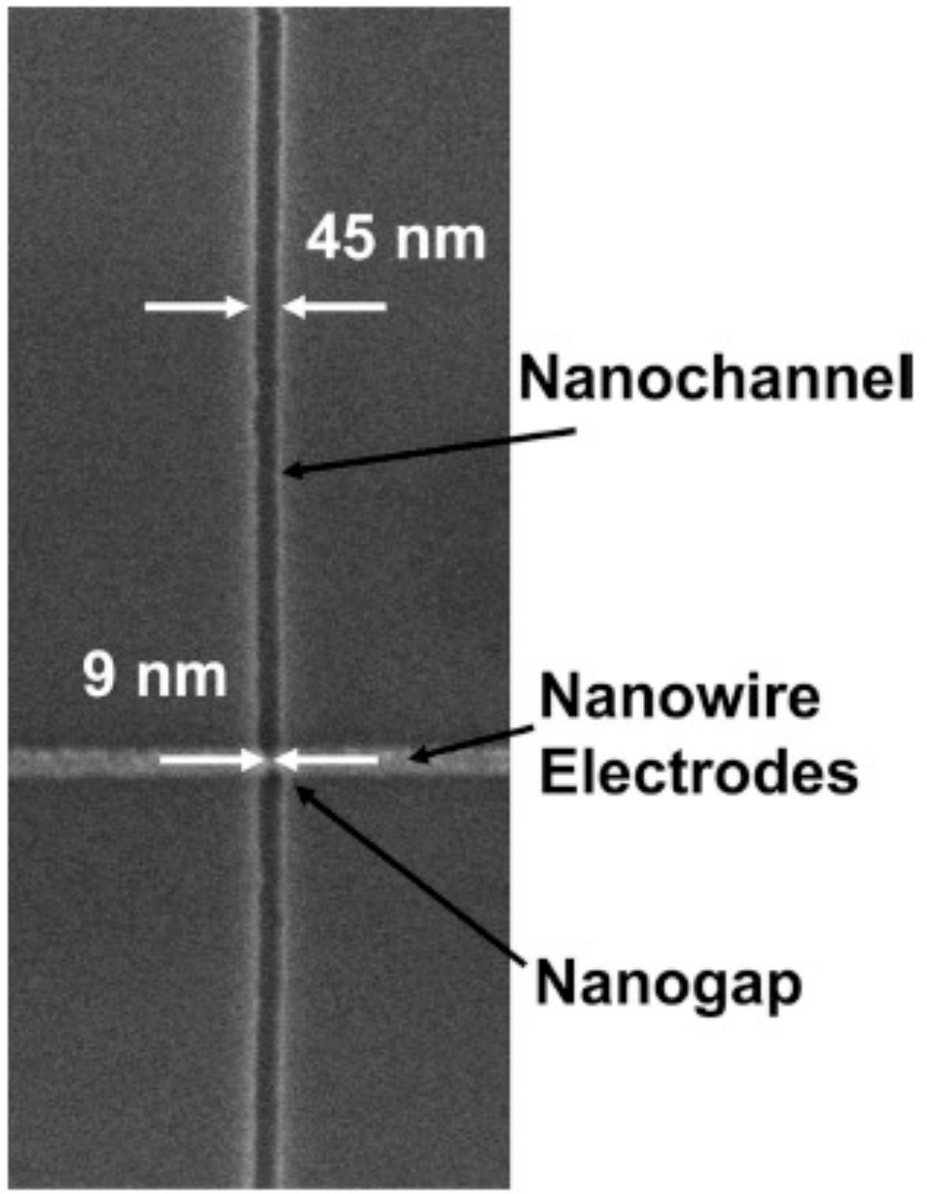
2) Water is a very wide band-gap semiconductor, "conduction" is via electrochemical ion neutralization (something the cold-fusion people have trouble with I think)

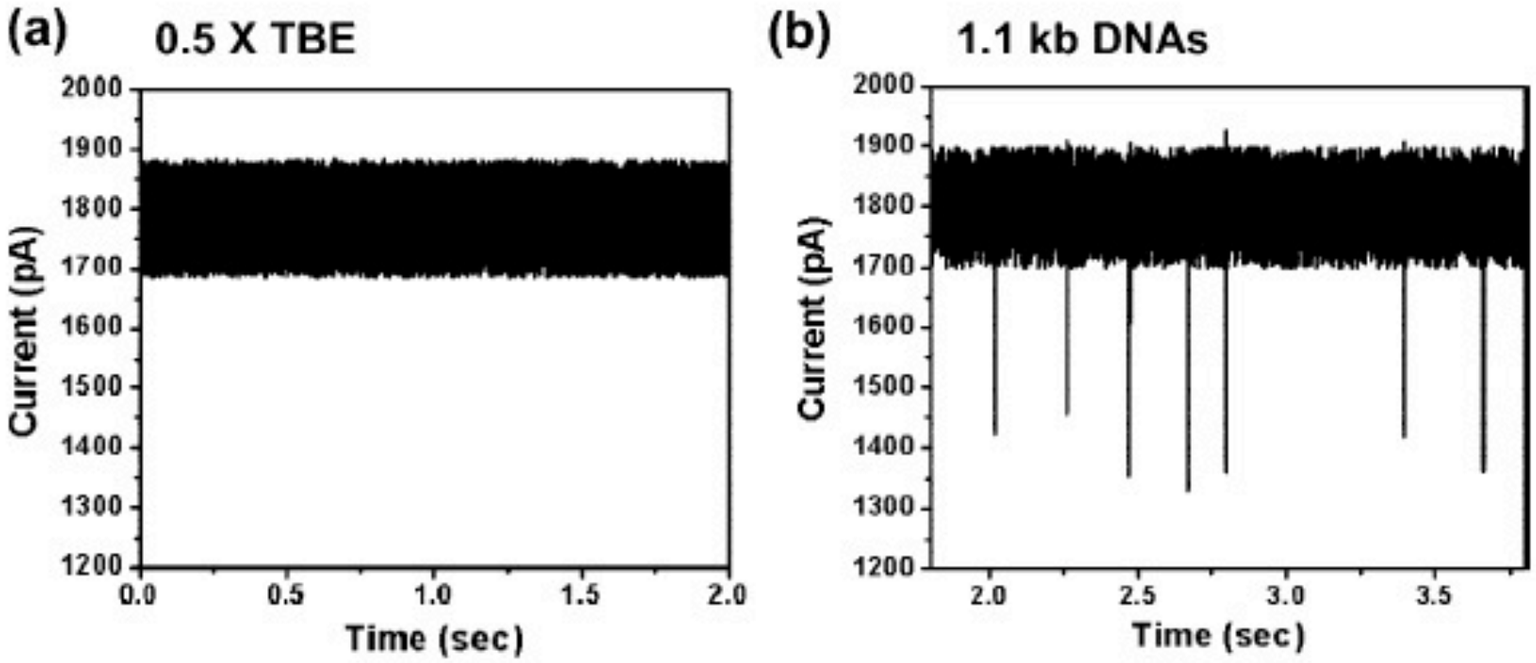
so: impedance is strictly capacitively coupled through the double-layer unless electrode potential reaches hydrolysis potential.

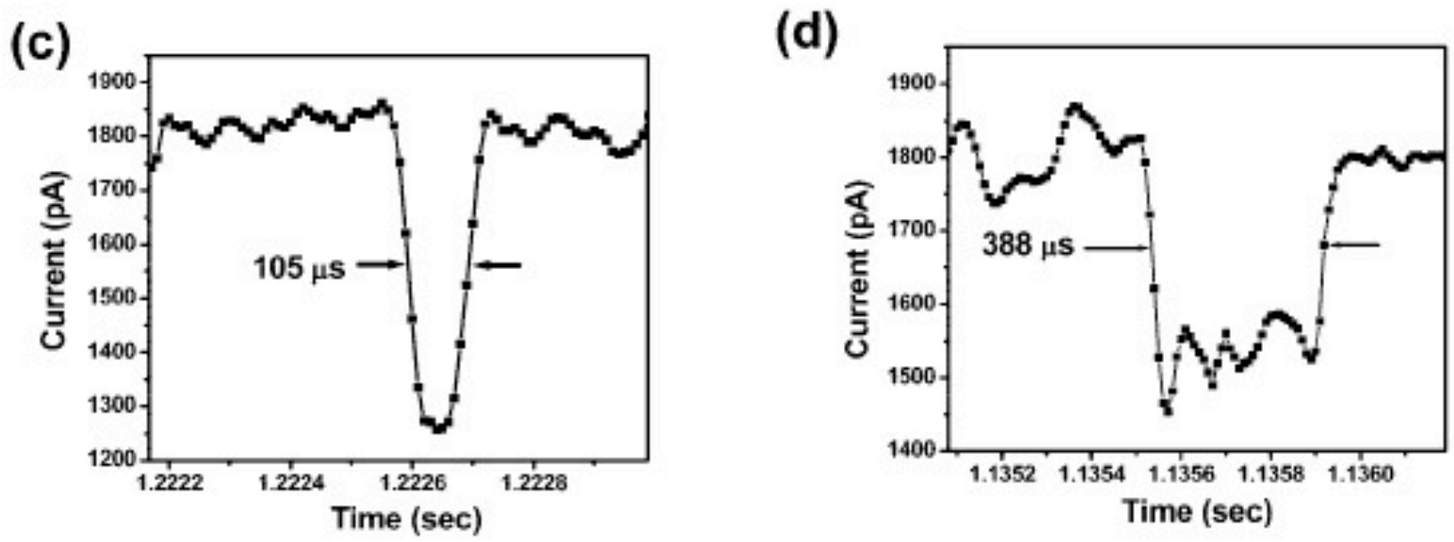
3) On-chip electronics (MOSFET?) probably needed for signal amplification/filtering/ multiplexing cannot withstand high temperature processing/high field sealing technologies, we need to develop "soft" nanochannel techniques.

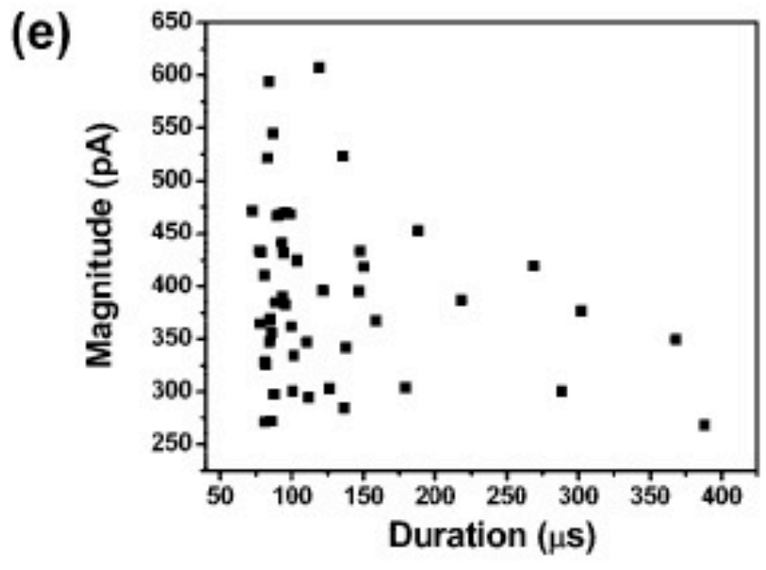
One of my Princeton colleagues jumped the gun on this I think:

X. Liang and S. Y. Chou, Nanogap detector inside nano-fuidic channel for fast real-time label-free DNA analysis," Nano Lett., vol. 8, pp. 1472-1476, 2008.

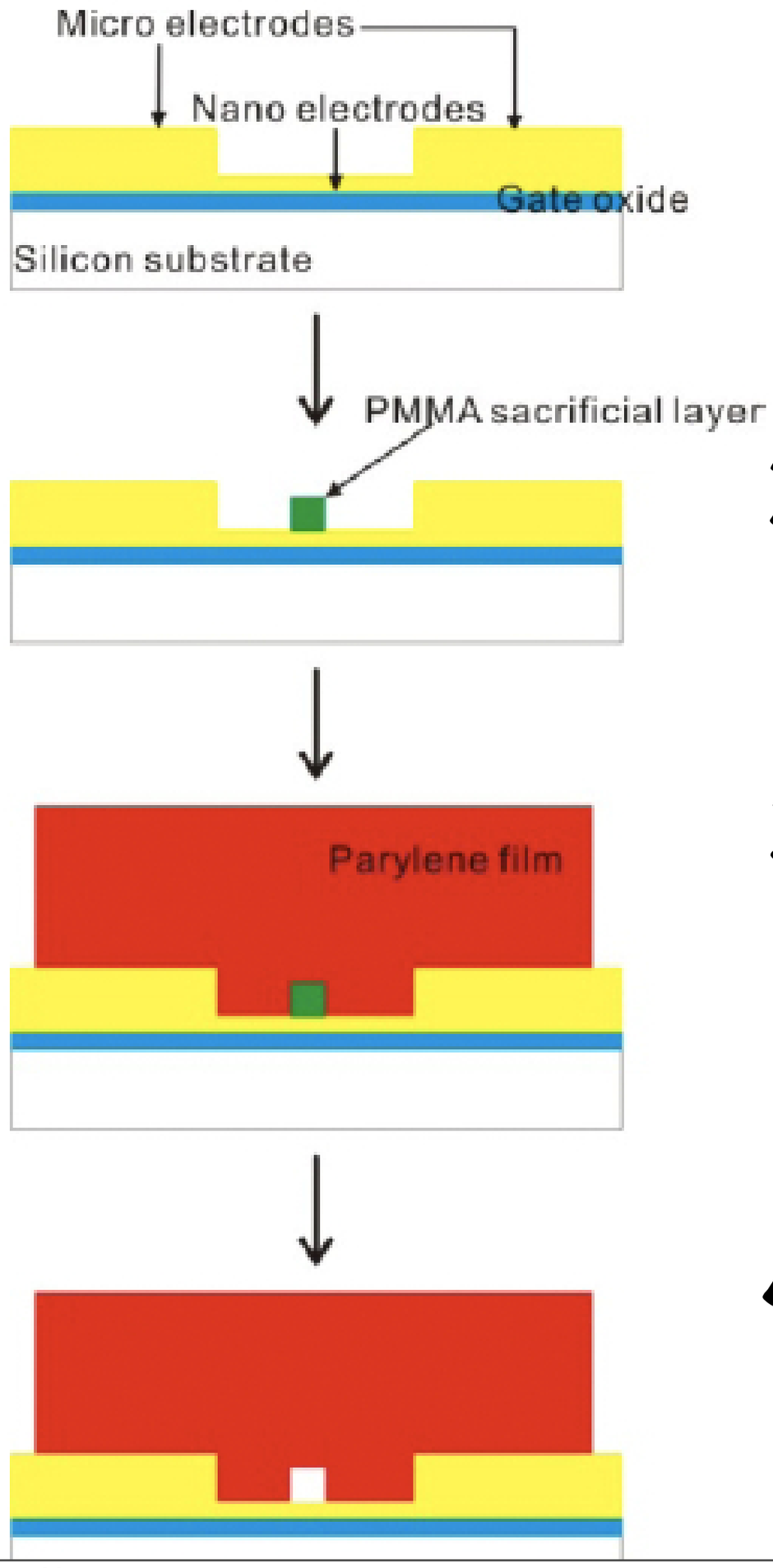



(a)
0.5 X TBE
Current (pA)
Time (sec)
(b)
1.1 kb DNAs
Current (pA)
Time (sec)


(c)
105 µs
Current (pA)
Time (sec)
(d)
388 µs
Current (pA)
Time (sec)


(e)
Magnitude (pA)
Duration (µs)

Our attempt to make an AC-coupled conformal system with low temperature processing for on-chip electronics.

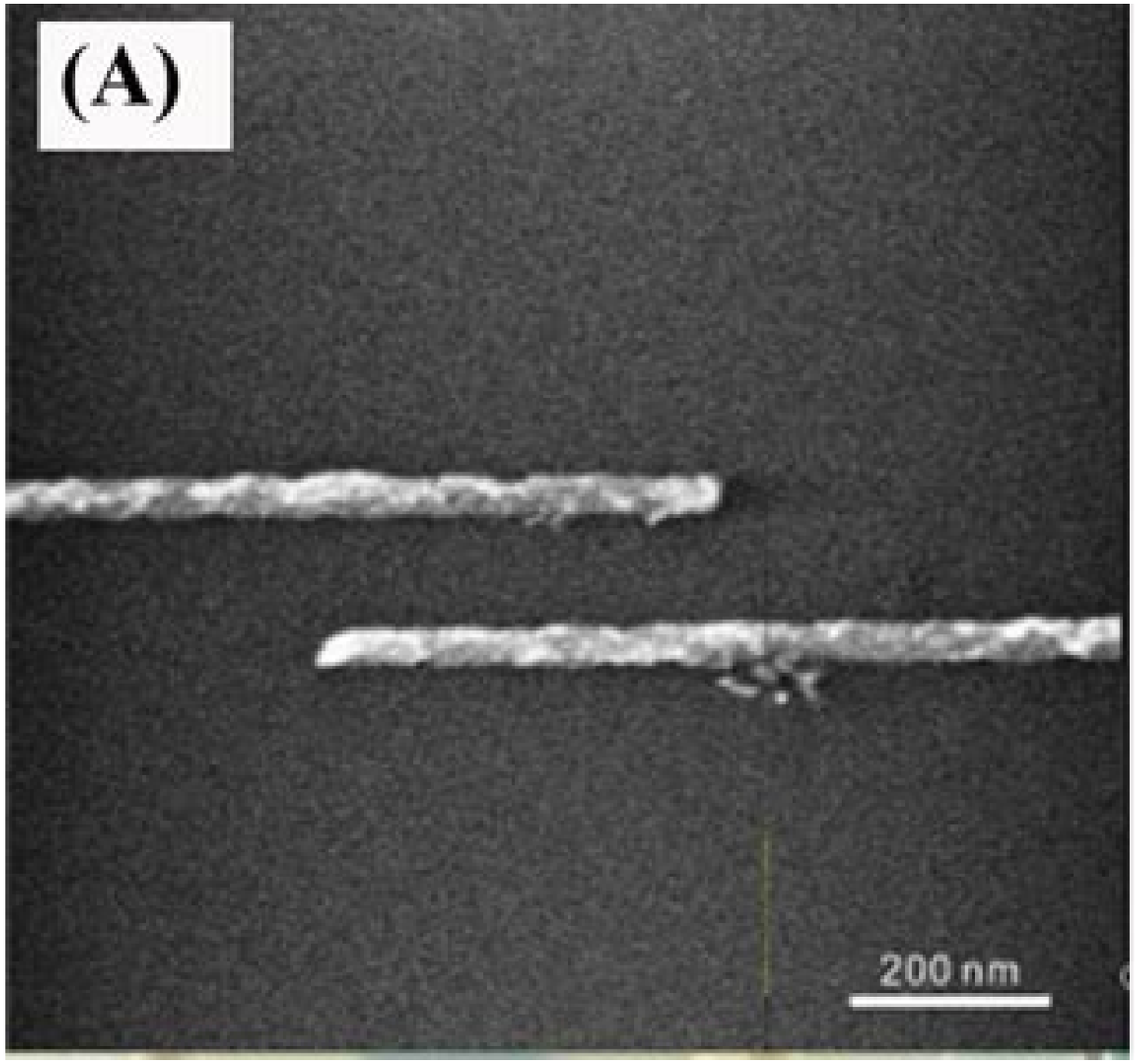


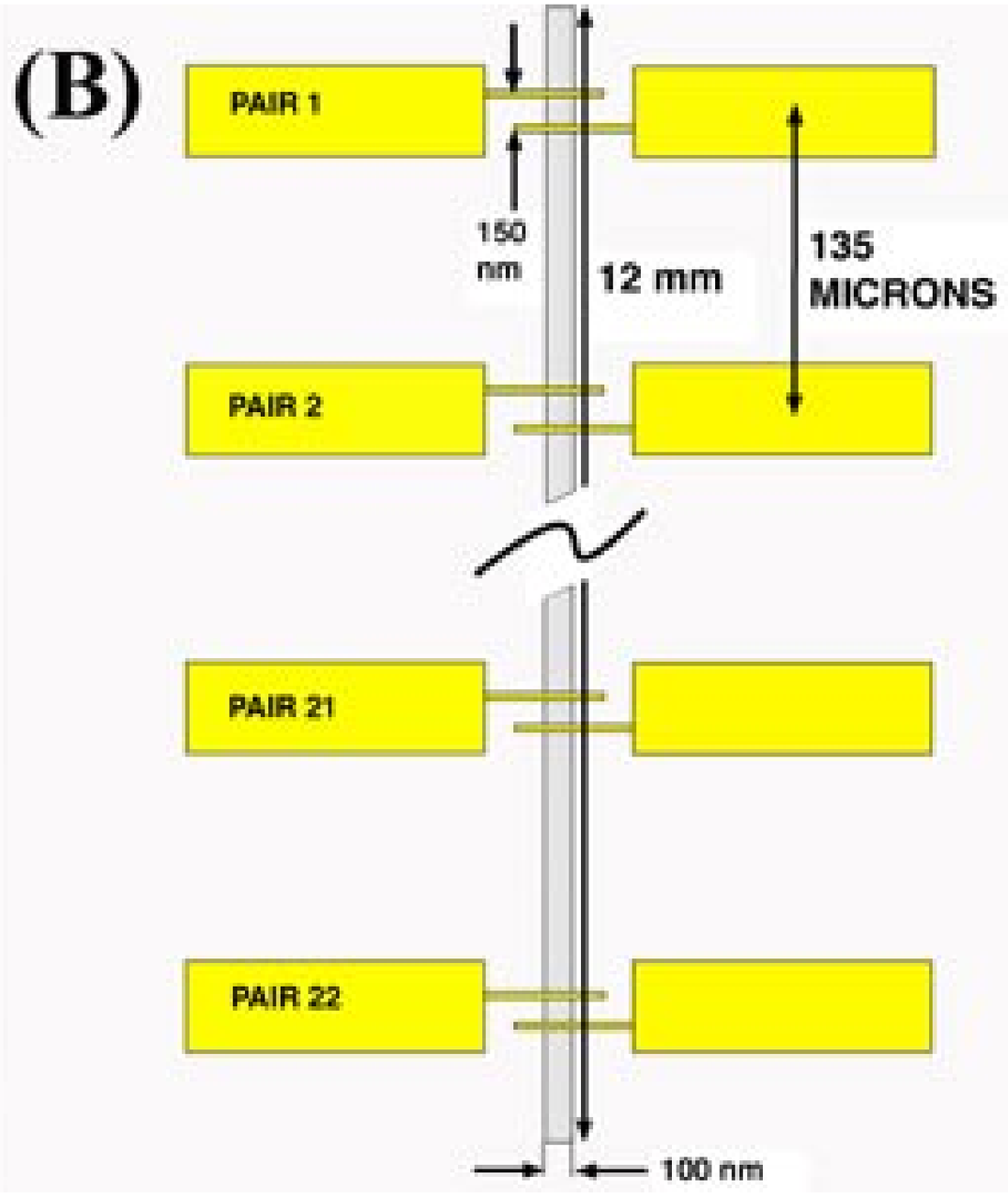
1) Electron-beam lithography (EBL) to make nanoelectrodes

2) EBL to make sacrificial PMMA nanochannels

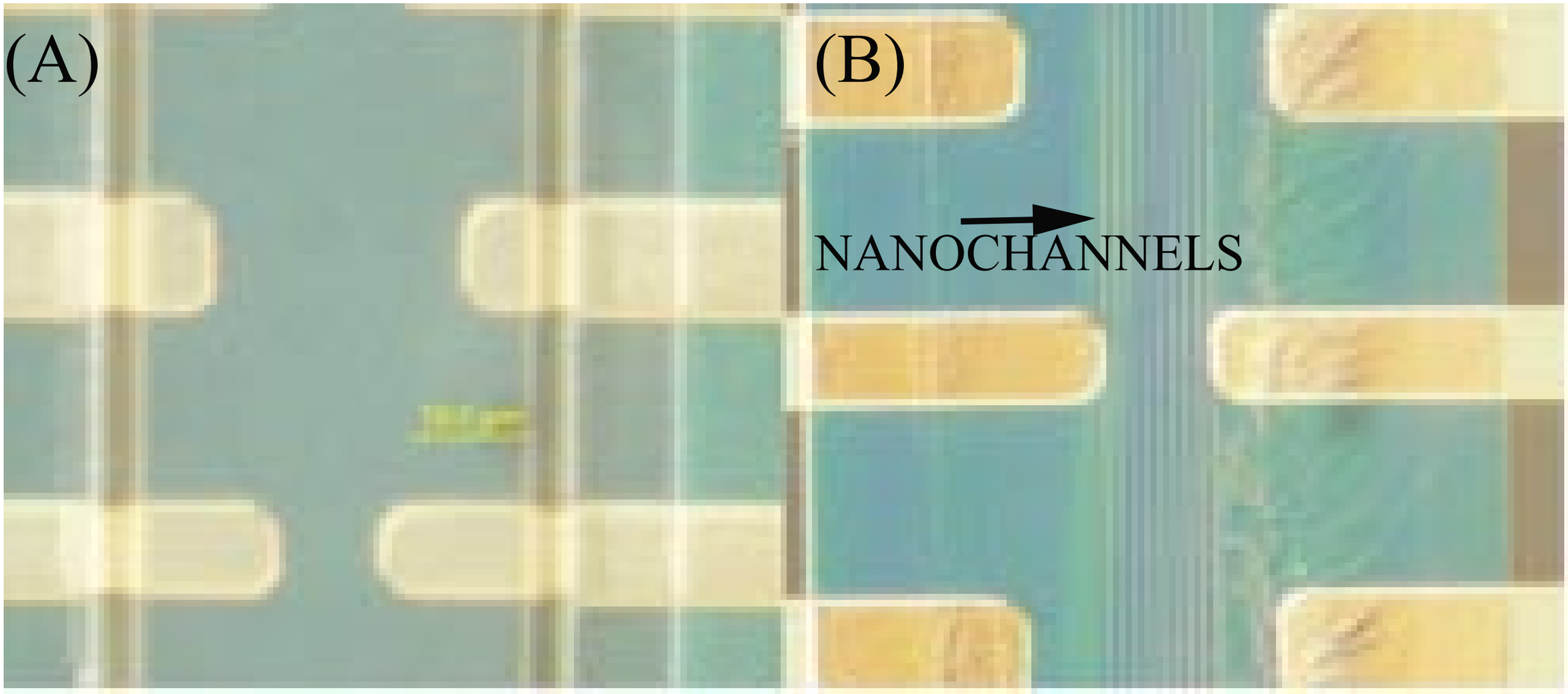
3) parylene-C to make conformal coating of PMMA nanochannels.

4) Sacrificial removal of PMMA

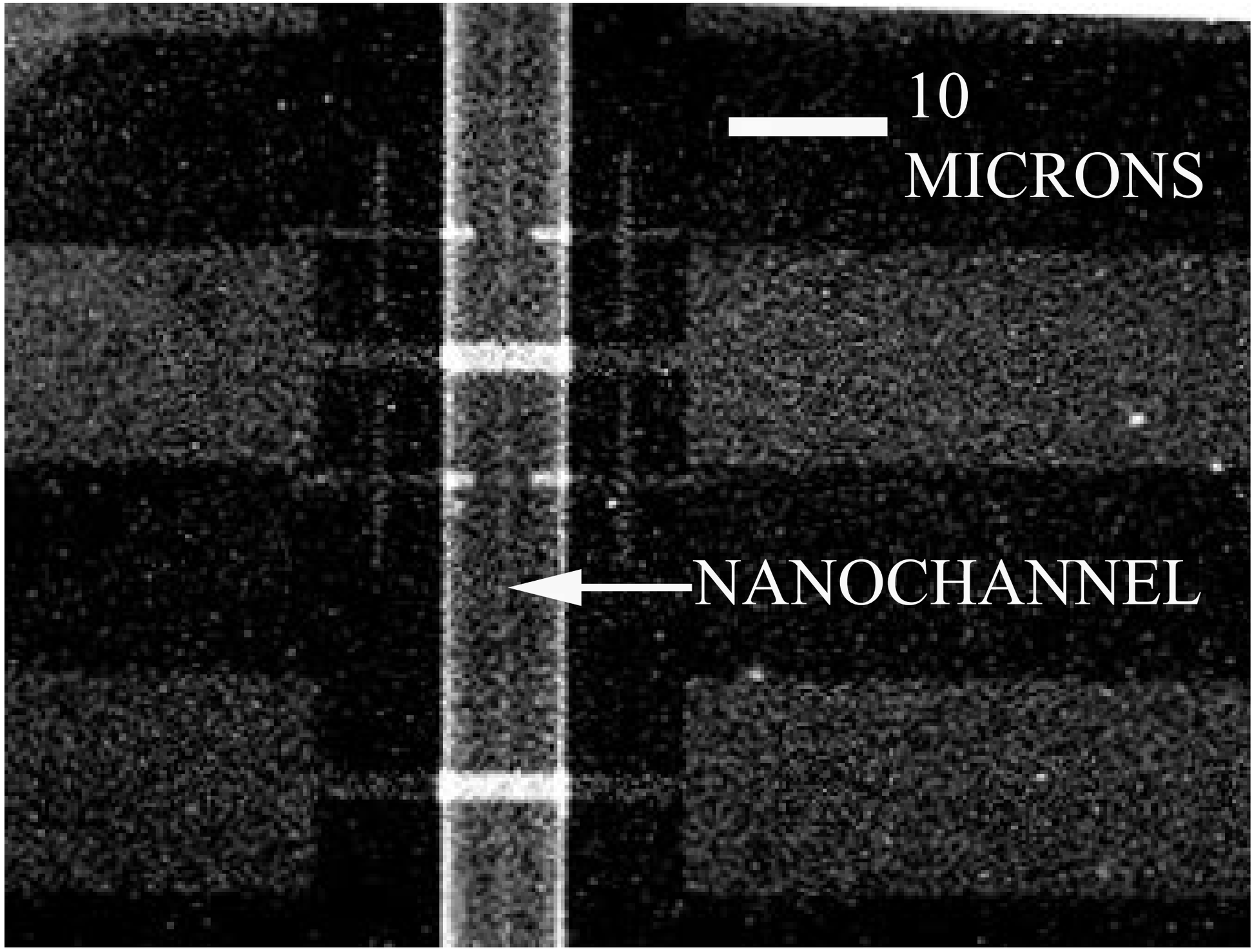




22 pairs of nanoelectrodes along a 12 mm long nanochannel

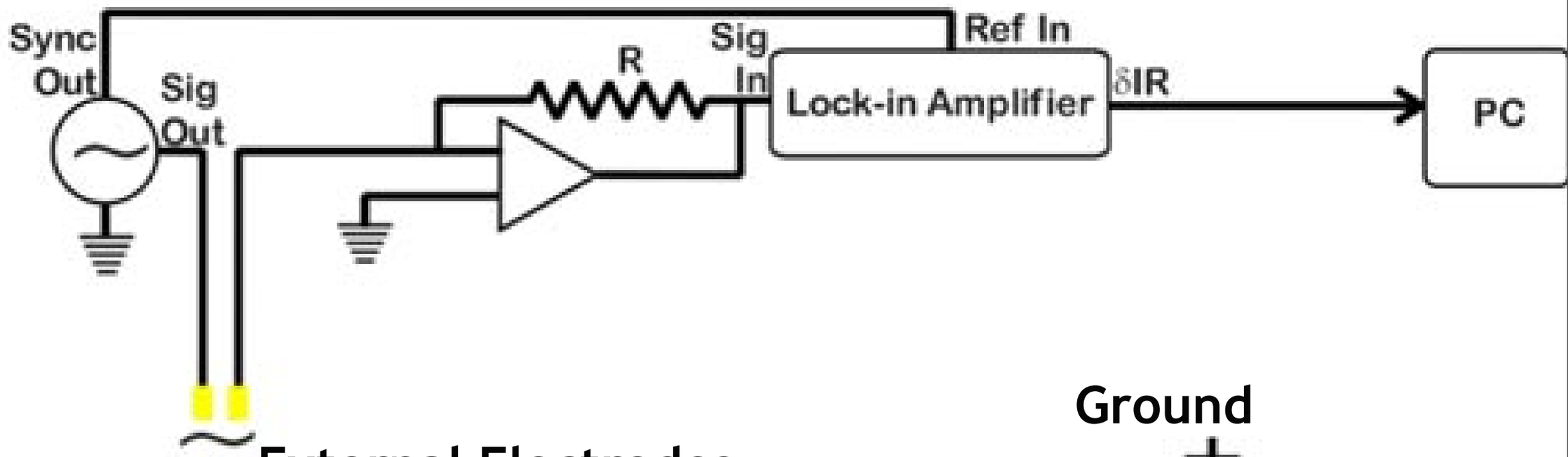


10 nm of SiO_2 to make hydrophyllic surface, wet easily (100 nm wide nanochannels)

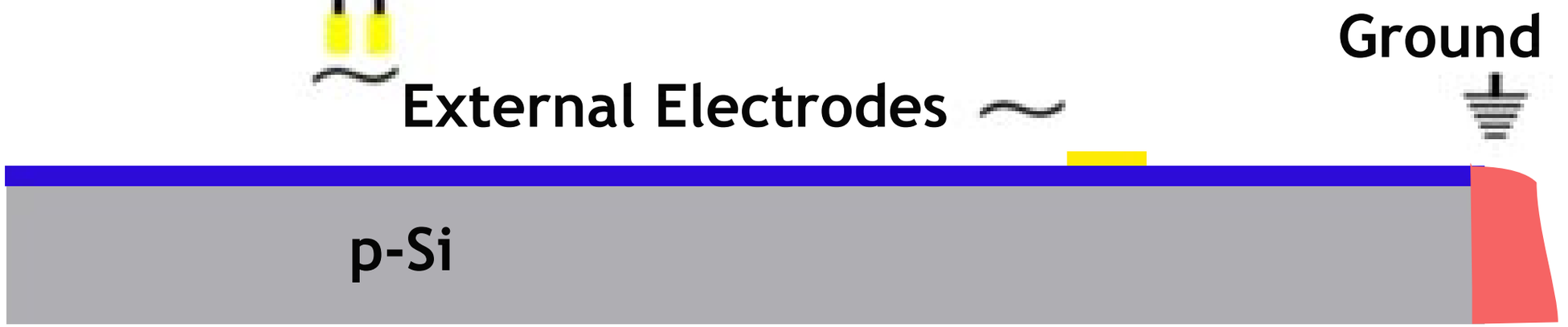


parylene C autofluorescence precludes single-molecule detection optically.

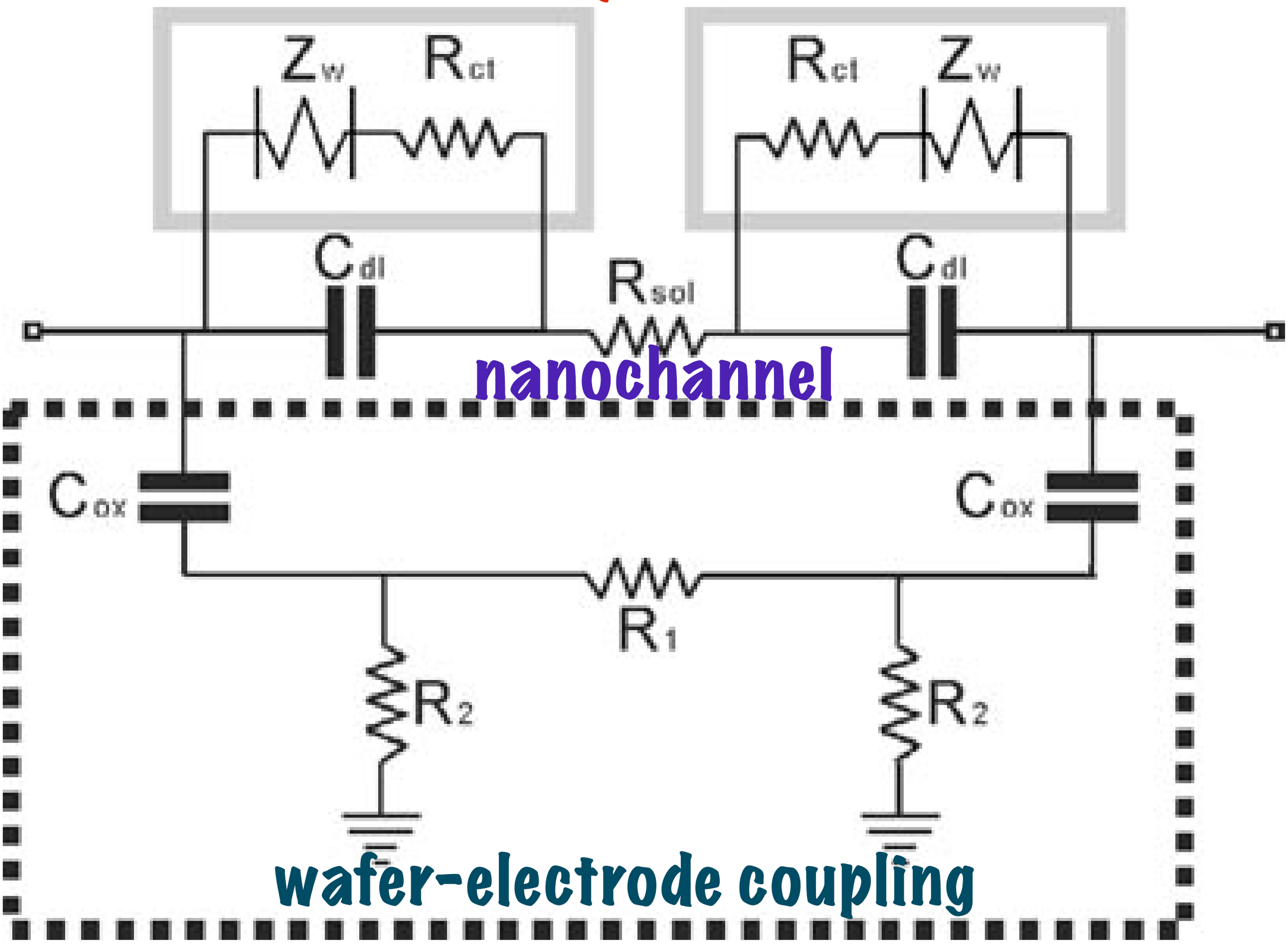
(A)



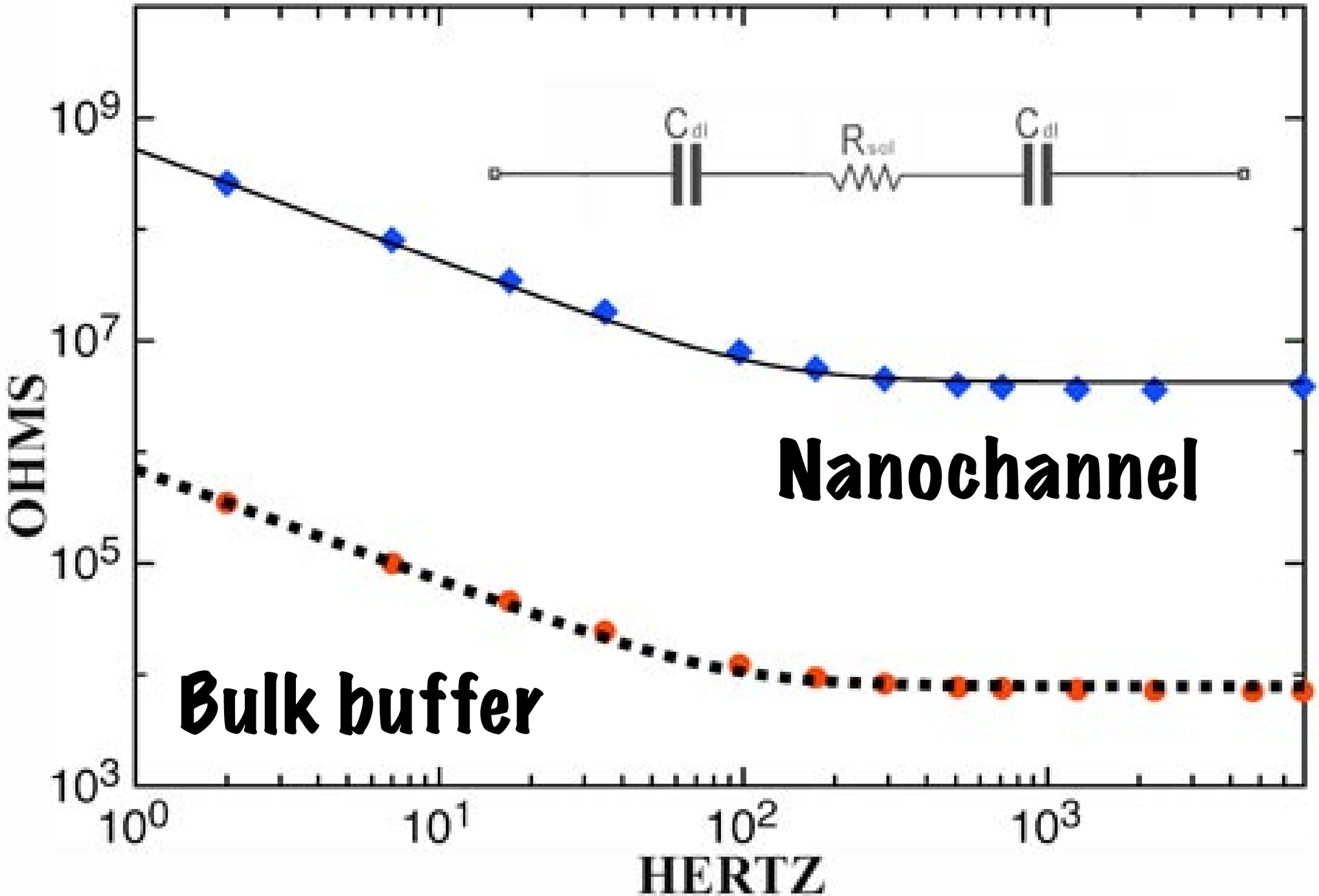
(B)



1) Nanoelectrodes fabricated on 100 nm of SiO_2 grown on p-doped Si wafer for ultimate FET operatation.
2) 10 mV RMS drive, no DC bias.


electrolysis channel
Zw
Rct
Rct
Zw
Cdl
Rsol
Cdl
nanochannel
Cox
Cox
R1
R2
R2
wafer-electrode coupling

Surprisingly, we understand some aspects of this complex circuit pretty well.

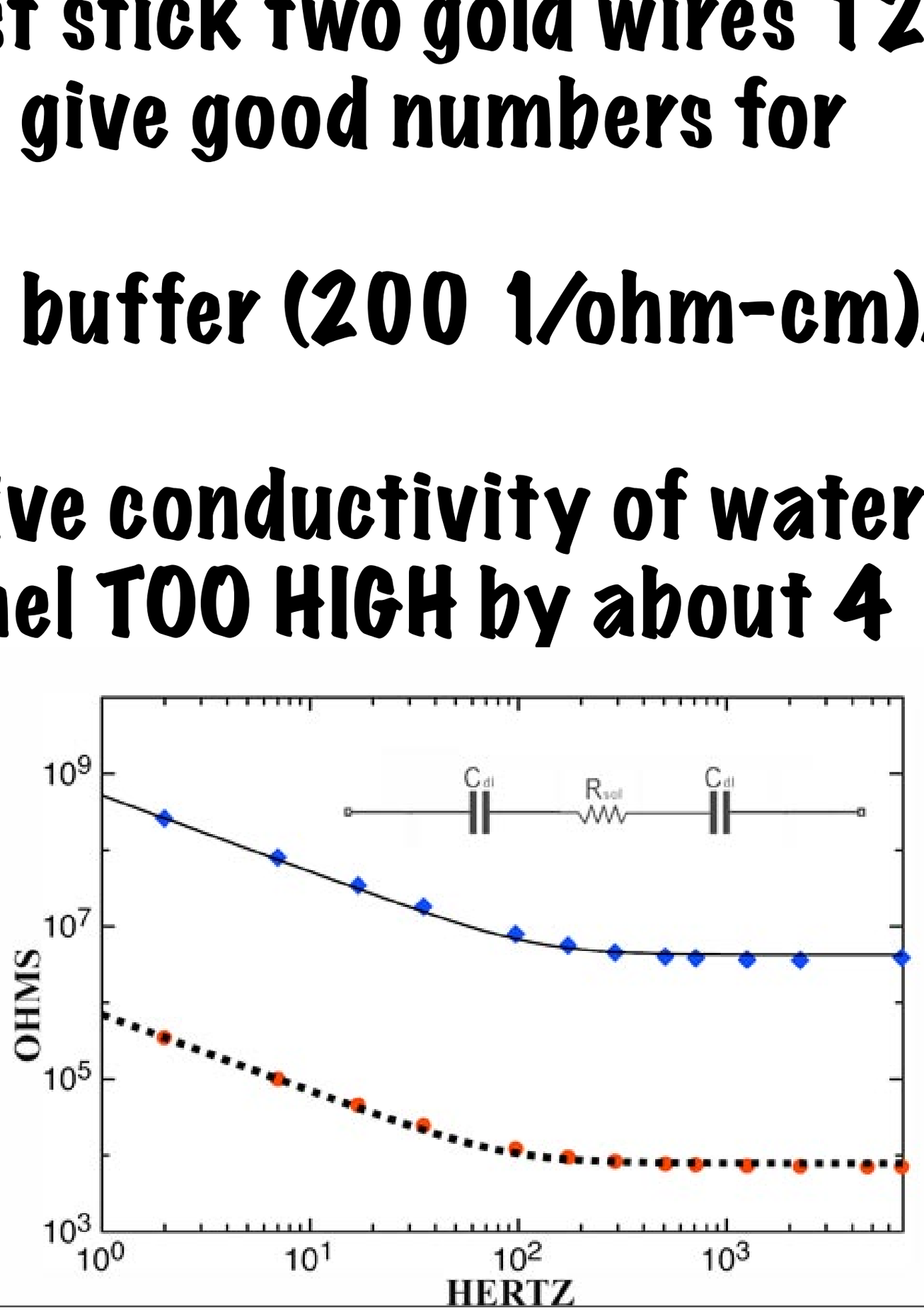


Longitudinal Impredance (like a nanopore).

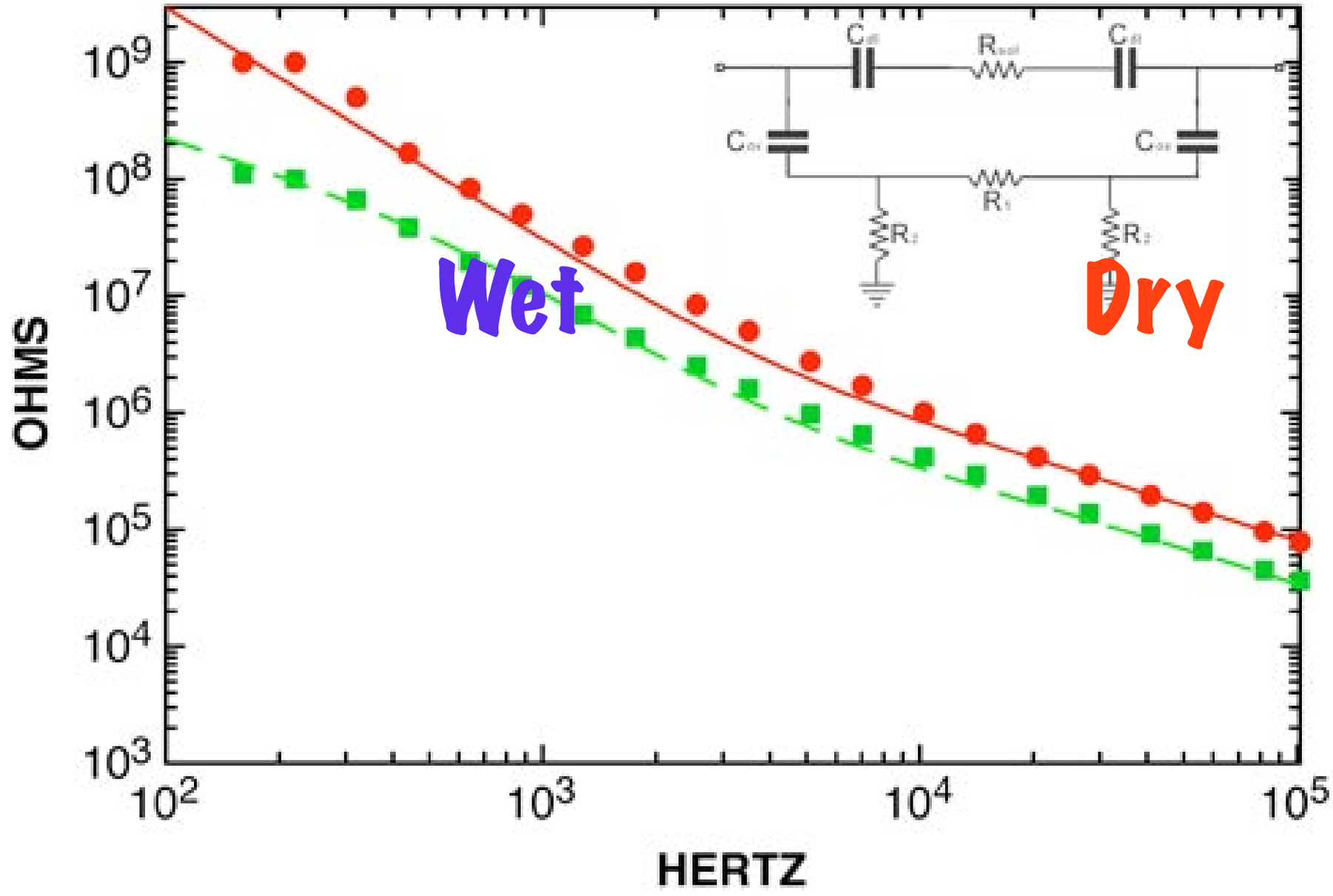
Fits to bulk water (just stick two gold wires 12 mm apart into buffer) give good numbers for C_{DL} (10 uF/cm^2) and conductivity of saline buffer (200 1/ohm-cm).

Fits to nanochannel give conductivity of water in 100 nm nanochannel TOO HIGH by about 4 orders of magnitude!

Big mystery right now.



Transverse impedance measurements between nanoelectrodes more complex because electrodes couple capacitively into p-dope Si wafer substrate of rather low resistivity of about 10 ohm-cm



1) Water barely visible in terms of impedance change compared to dry electrode because of capacitive coupling to substrate.

2) Still get impedance of solvent far too small by about 4 orders of magnitude.

3) DNA detection electronically with high speed HOPELESS with this configuration!

Mothers: don't let your children try to electronically detect DNA!

5. Quo vadis?

1) I think we have to construct nanochannels in front of nanopores in order to wring out the entropy and get control of the molecule pasing by the nanopore.

2) AC-couple transverse nanoelectrodes

Marija Drndic, U Penn Physics: transverse nanoelectrodes

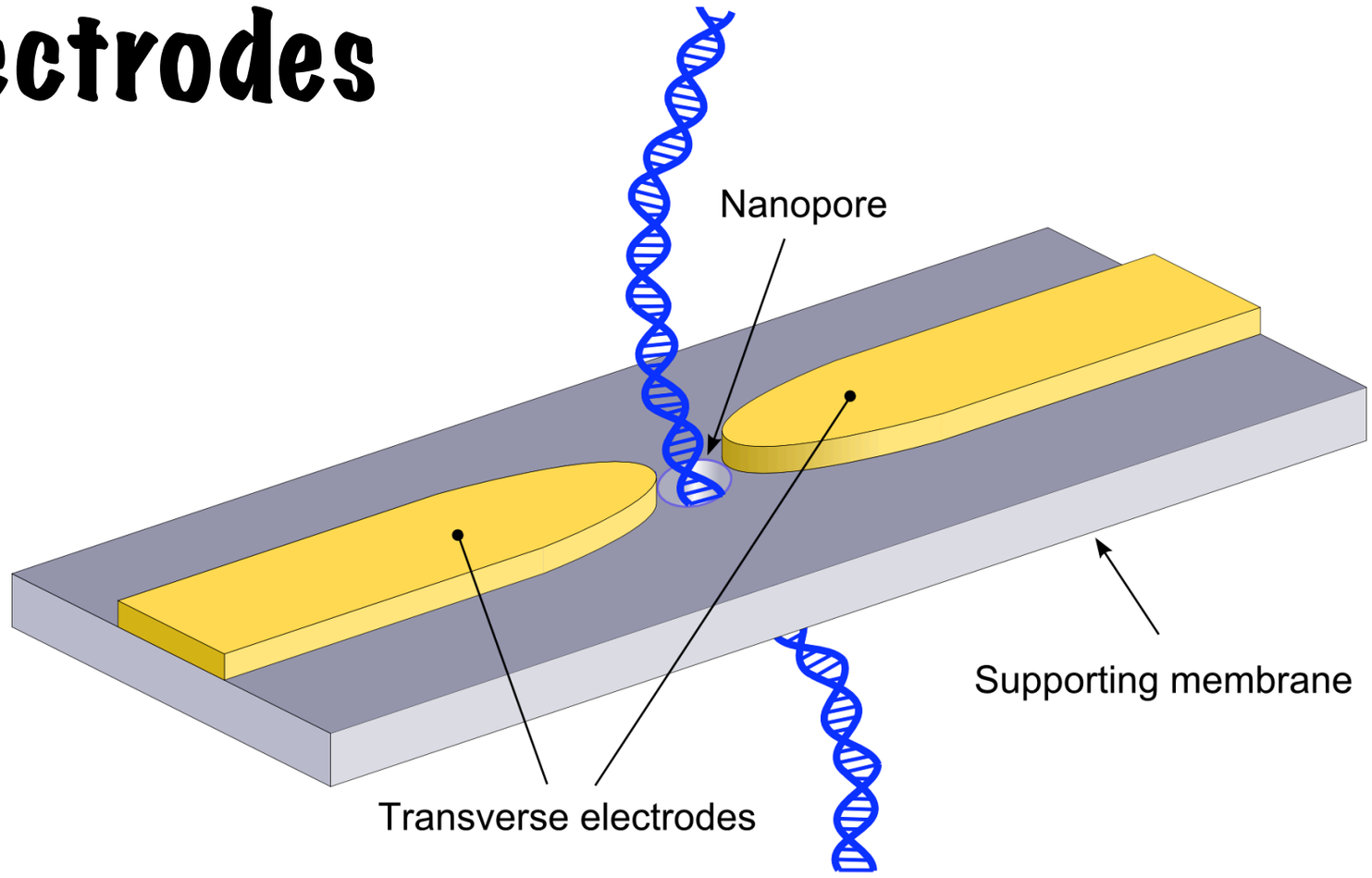
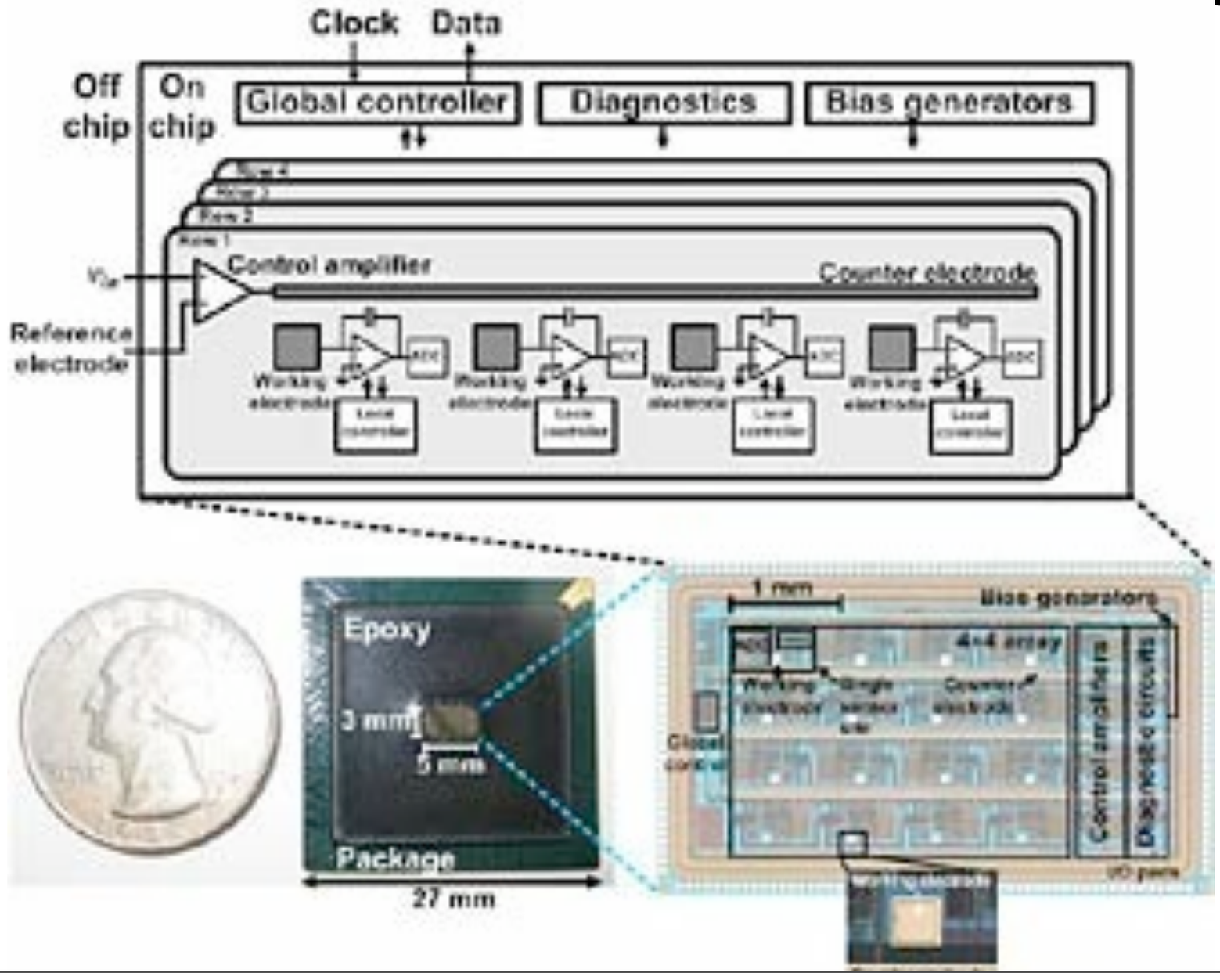


Figure 0. Diagram of a transverse electrode configuration, where both electrodes are positioned on the same membrane surface to sense across the pore aperture, intended to allow new sensing strategies such as measurement of tunneling current through translocating DNA molecules.

3) On-chip MOSFET amplifiers to locally amplify up expected tiny signals at the pA level at the MHz bandwidths.

Ken Sheppard, EE, Columbia University



We'll get there!

Thanks!