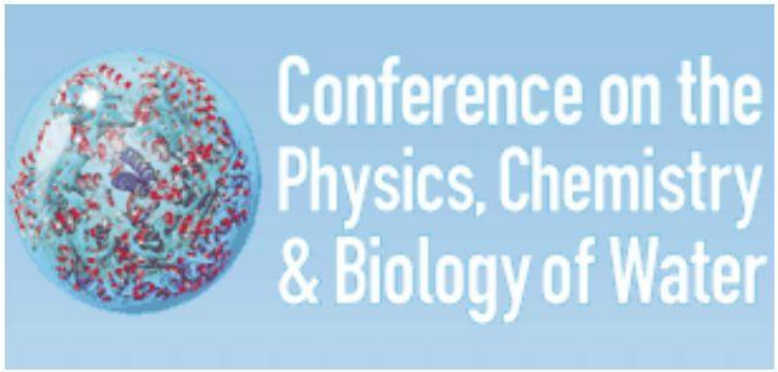
7th Annual



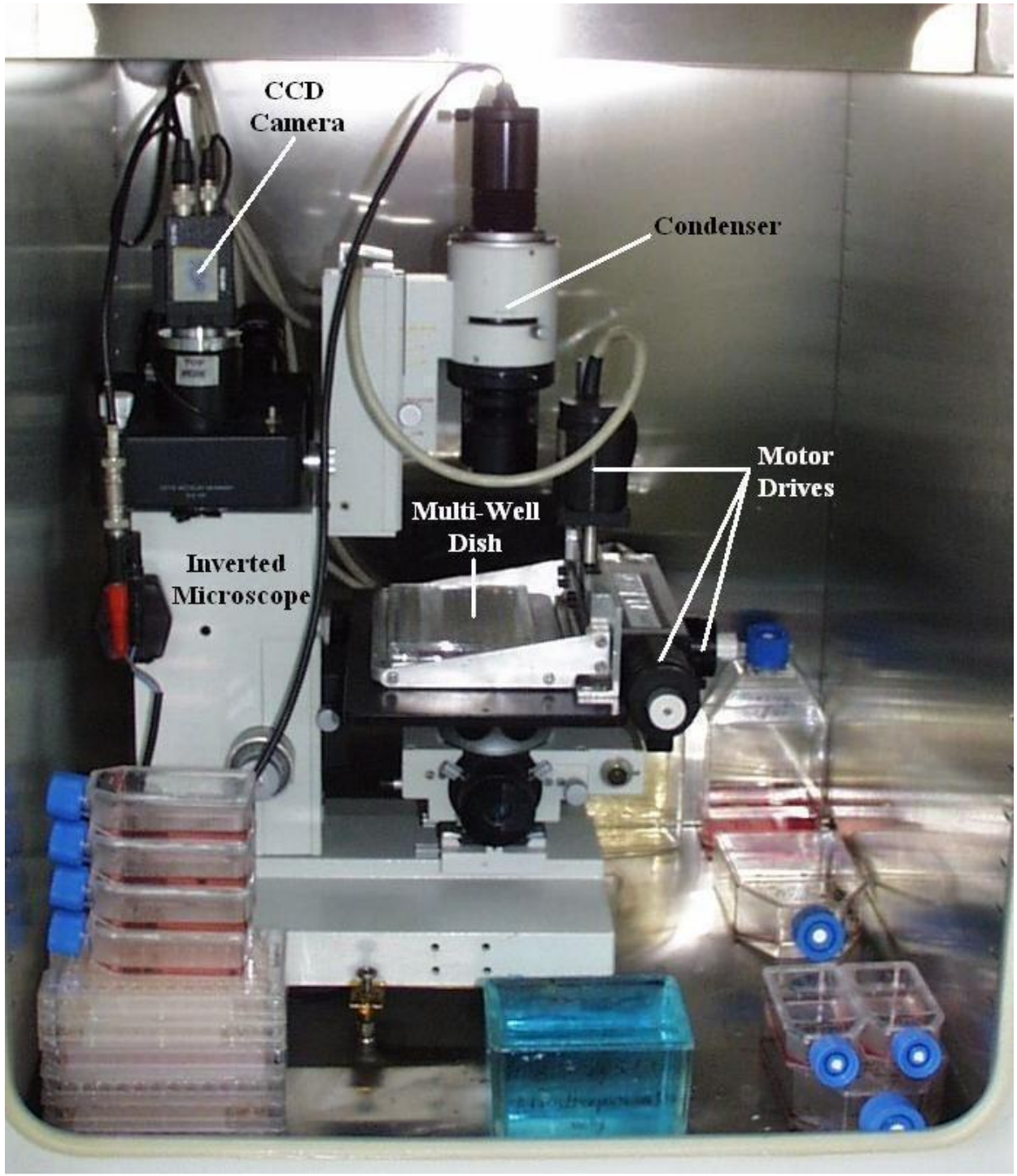
October 18th-21st, 2012

How Cancer Cells and Magnetic Fields led us to *Water*

Paul Héroux[1,2] and Ying Li[1]

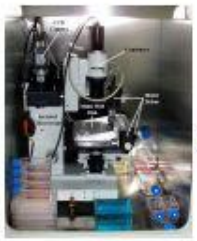
[1]InVitroPlus Laboratory, Department of Surgery, Royal Victoria Hospital, Montreal, Canada.
[2]Department of Epidemiology, Biostatistics and Occupational Health, McGill University, Montreal, Canada.

InVitro *Plus*


CCD
Camera
Condenser
Motor
Drives
Multi-Well
Dish
Inverted
Microscope

Microscopy and Analysis, 18 (3): 13-15 (UK), 2004.

Hardware



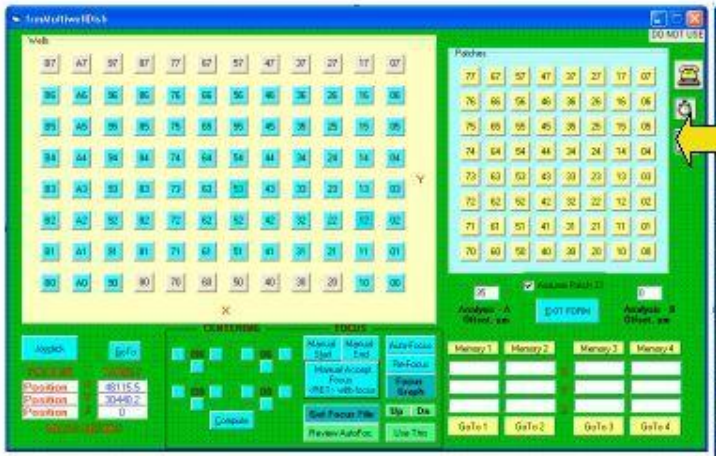
FrontEnd ⟷ Imaging ⟷ Recognition ⟷ Analysis

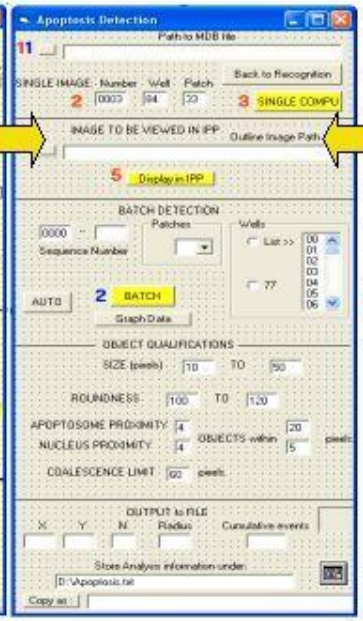
Function

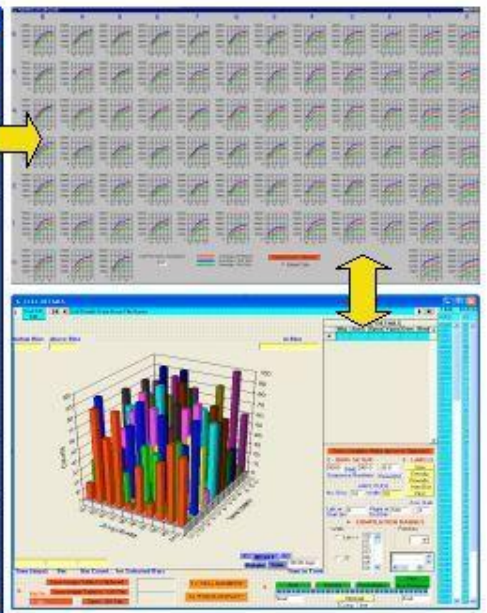
FrontEnd	Imaging	Recognition	Analysis
Move multi-well dish. Acquire images.	Find objects within images. Build database files.	Show recognition performance to operator.	Graphs of proliferation and apoptosis. Display: custom graphs.

Software

FrontEnd.exe ⟷ Imaging.exe ⟷ Recognition.exe ⟷ Analysis.exe & Display.exe



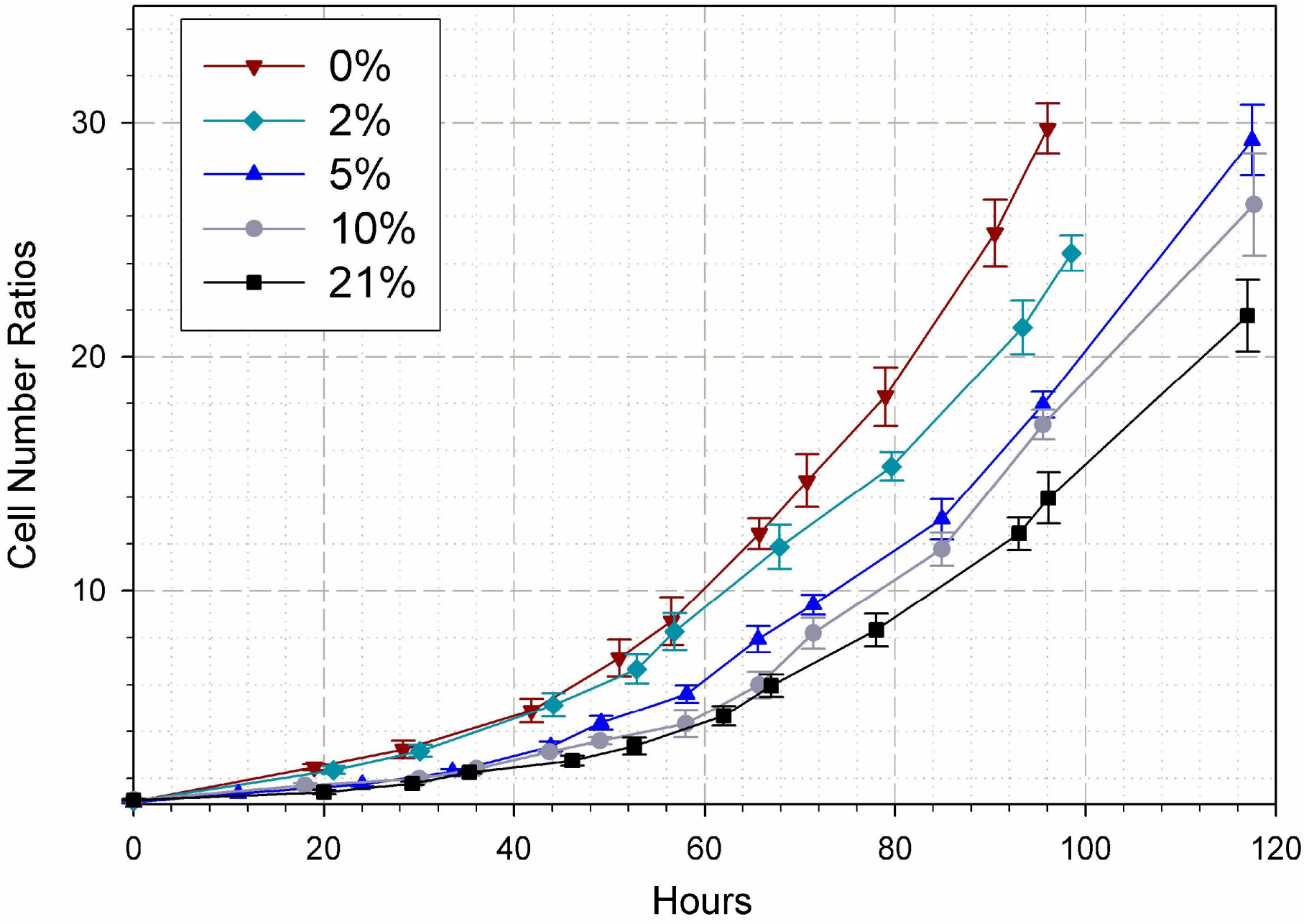




Initial findings:

Levels of Oxygen over the Cell Cultures influence

- Cell Proliferation
- Metastasis
- Karyotype


0%
2%
5%
10%
21%
Cell Number Ratios
30
20
10
0
20
40
60
80
100
120
Hours

Karyotype Contraction

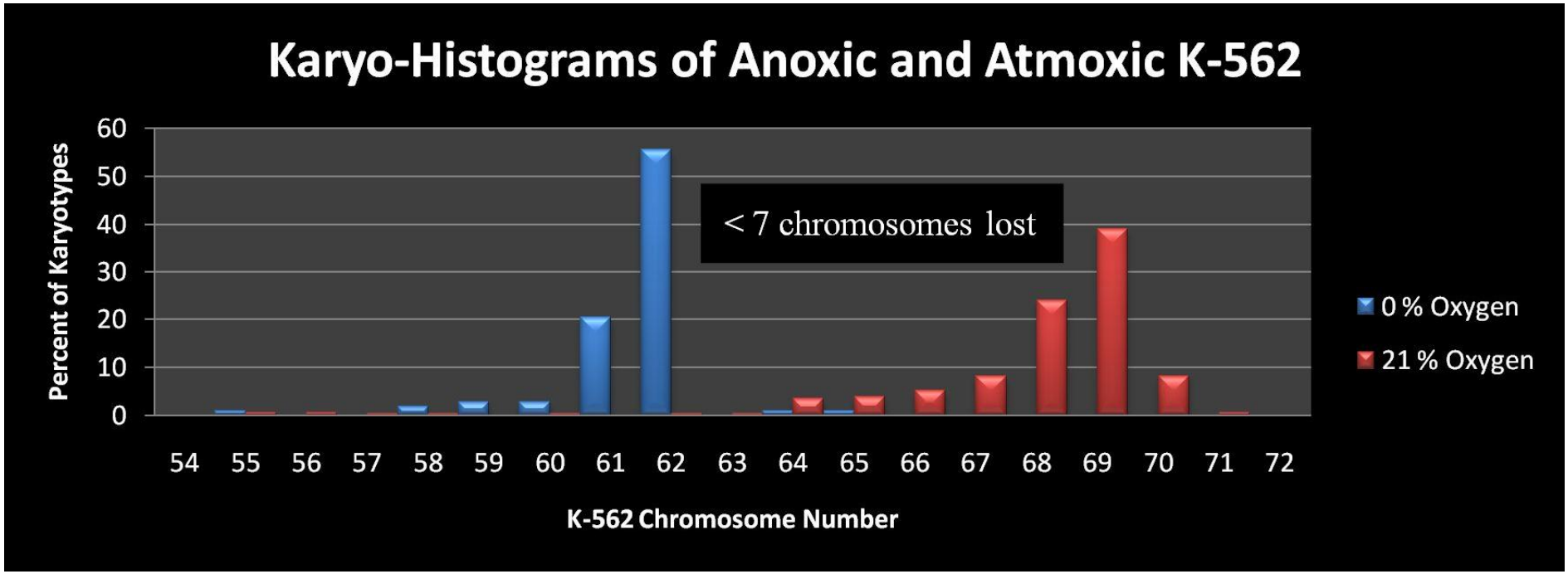
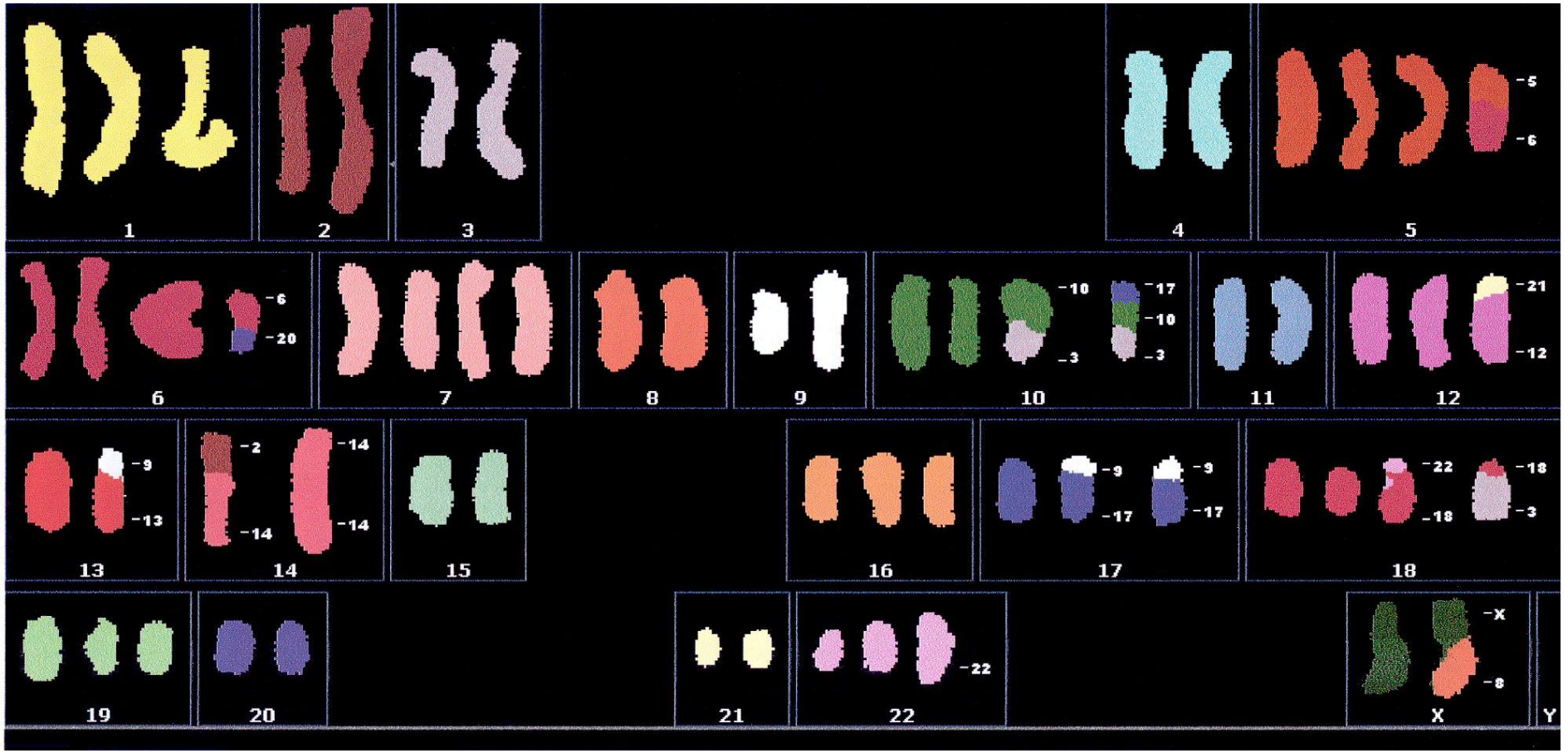

Karyo-Histograms of Anoxic and Atmoxic K-562
Percent of Karyotypes
60
50
40
30
20
10
0
< 7 chromosomes lost
54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72
K-562 Chromosome Number
0 % Oxygen
21 % Oxygen

Table 5. Karyotype Contractions in Cancer Cell Lines after 3-day Metabolic Restrictions.

		Atmoxia Baseline	Anoxia Alone	Atmoxia		
Cell	Type	Mode (80 % Range)	Mode (80 % Range)	Oligomycin 0.1 µM[4] Mode (80 % Range)	Imatinib 0.08 µM[5] Mode (80 % Range)	Melatonin-Vit C 0.3 µM, 150 µM[6] Mode (80 % Range)
K562[1]	Erythro-Leukemia	69 (64-70)	62 (58-62)	48 (46-53)	47 (45-51)	48 (45-52)
HEL[2] 92.1.7	Erythro-Leukemia	66 (62-67)	59 (57-60)	47 (46-51)	48 (47-53)	49 (46-52)
NCI-H460	Large Cell Lung Cancer	57[3] (53-65)	51 (45-52)	47 (46-49)	47 (46-50)	47 (45-51)
COLO 320DM	Colo-rectal Adeno-carcinoma	54[3] (49-61)	48 (46-49)	46 (46-48)	46 (45-48)	47 (45-49)
MCF7	Breast Adeno-carcinoma	82[3] (66-87)	74 (61-75)	64 (59-66)	65 (61-68)	63 59-65

Number of metaphases for each determination: 122, 108, 30, 25, 25; 50, 50, 30, 25, 20; ATCC, 20, 20, 20, 20; ATCC, 50, 20, 20, 20; ATCC, 35, 20, 25, 30. [1] BRC-ABL positive. [2] BRC-ABL negative. [3] ATCC data. [4]Proliferation IC50 of 0.0125 µM for K562 and HEL IC50 of 0.1 µM for other types. [5]K562 sub-toxic level. [6]The Melatonin-Vitamin C concentrations optimized for K562 chromosome count normalization.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-14
15
16
17
-9
-17
-9
-17
18
-22
-18
-18
-3
19
20
21
22
-22
X
-X
-8
Y

Karyotype Contraction:

the reversible loss of chromosomes by a cancer cell.

The majority of cancer cells are hyperploid: they have more than 46 chromosomes.

If metabolism is suppressed, the number of chromosomes decreases.

If metabolism is increased, the number of chromosomes rises.

Table 5. Karyotype Contractions in Cancer Cell Lines after 3-day Metabolic Restrictions.

		Atmoxia Baseline	Anoxia Alone	Atmoxia		
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COLO 320DM	Colo-rectal Adeno-carcinoma	54[3] (49-61)	48 (46-49)	46 (46-48)	46 (45-48)	47 (45-49)
MCF7	Breast Adeno-carcinoma	82[3] (66-87)	74 (61-75)	64 (59-66)	65 (61-68)	63 59-65

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Metabolic Suppression and Cancer

Agents capable of suppressing metabolism reduce chromosome numbers in cells, and may increase the rate of cancer growth because…

- **they enhance glycolysis (alternate metabolism),**
- **they suppress detoxification mechanisms, and**
- **fewer chromosomes are more easily duplicated.**

Clinicians say that hypoxic tumors are more malignant.

Epidemiological evidence points to deleterious effects of anti-oxidants administered to cancer patients (vitamin A on lung cancer rates in smokers).

RESEARCH ARTICLE

Tumor Biology 33(1), pp. 195-205, 2012

Metabolic restriction of cancer cells in vitro causes karyotype contraction—an indicator of cancer promotion?

Ying Li · Paul Héroux · Igor Kyrychenko

Received: 8 August 2011 / Accepted: 27 October 2011

Abstract The metabolism of cultured cancer cells is stimulated by 21% oxygen and generous nutrition, while real tumors grow in oxygen and nutrient-restricted environments. The effect of these contrasted conditions was studied in five hyperploid (54–69) cancer cell lines. When grown under anoxia and in the presence of antioxidant metabolic restrictors, the cell lines quickly reverted to almost normal chromosome numbers (47–49). The stepped withdrawal of oxygen over K562 showed progressive increases in proliferation rate and the acquisition of a stable, stem phenotype. In genetic studies, hyperploid cancer cells adjusted their chromosome numbers up or down to match their micro-environment through rapid mechanisms of endo-reduplication or chromosome loss. These fast reactions may explain the surprising adaptability of tumor cells to oncologic interventions. Furthermore, karyotype contraction may provide a basis for the previously observed carcinogenic influence of the administration of some antioxidants in human populations.

Magnetic Fields


B-FIELD SENSOR
PROBE
REMOTE
B 5.897uT
FREQUENCY: 481 Hz
10uT AUTO
RMS
LIVE
50Hz, 32kHz
F1
F2
F3
F4
ON
ESC
SAVE
CHARGE
RUN
ENTER
ALARM
USER
MEM
CONF
MODE
narda
EFA-300

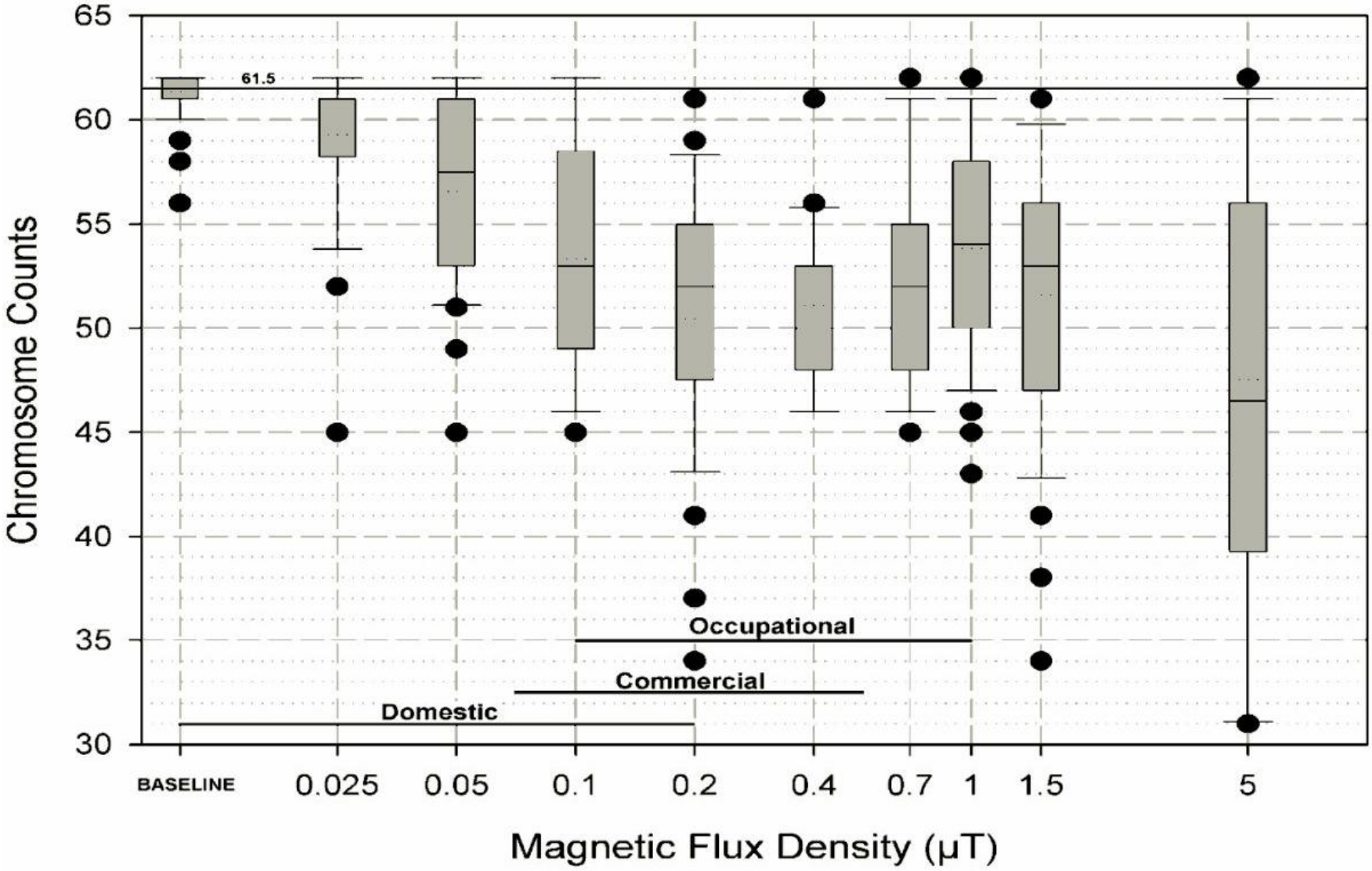


Fig. 3.14: K562 chromosome counts as a function of 60-Hz Magnetic Flux Density applied for 6 days to naïve cells. In sequence, 65, 28, 50, 77, 46, 33, 65, 102, 56 and 50 metaphases. Approximate ranges for typical domestic, commercial and occupational exposures are indicated.

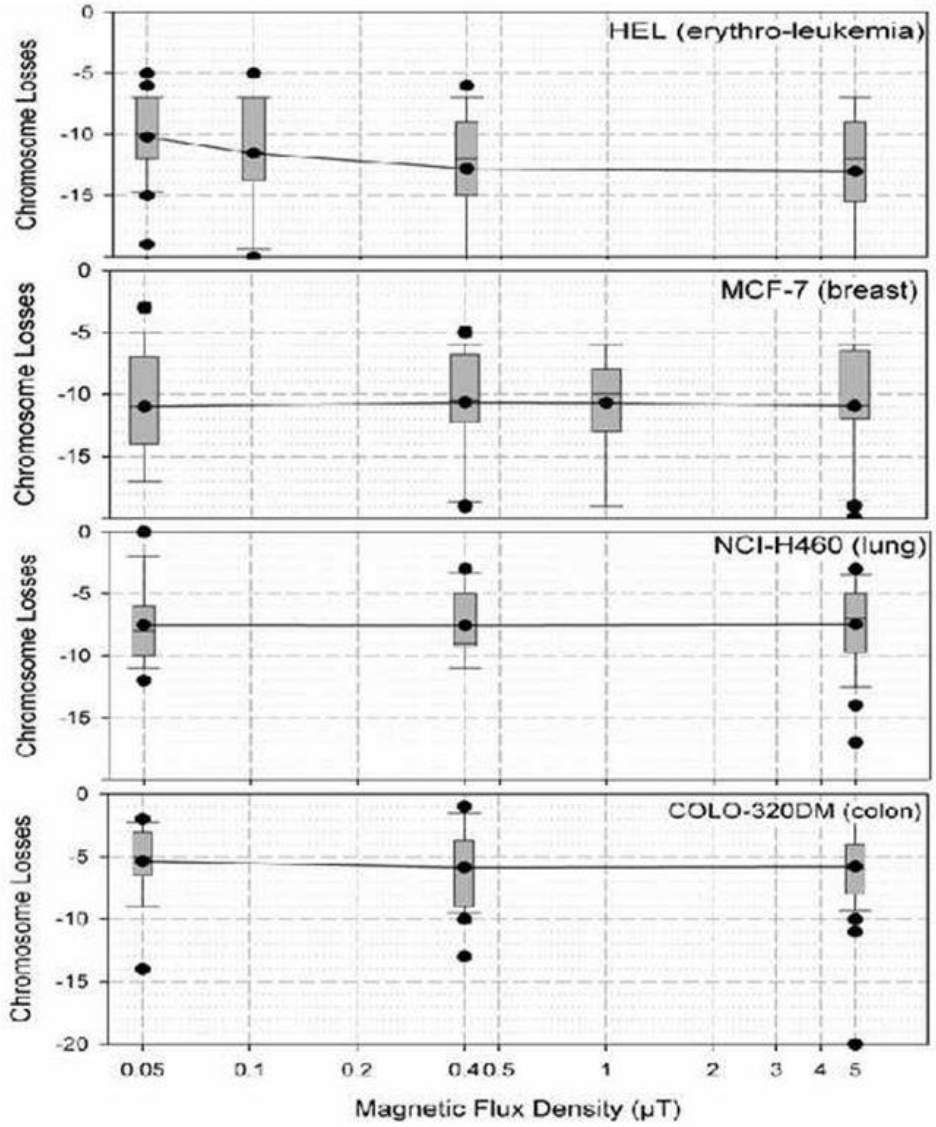


Fig. 3. Average chromosome losses in erythro-leukemia, breast, lung and colon cancer cells as a function of 60-Hz Magnetic Flux Density. The chromonome number baseline averages for naïve cells ("0"), 80 % range and metaphase number (< 4 nT) are: HEL: 66, 62-67, 32; MCF7: 74, 61-75, 30; NCI-H460: 57, 53-65, 30 and COLO 320DM: 54, 49-61, 30. 6-day assays with, in sequence, 32, 22, 29, 32; 19, 22, 19, 21; 29, 22, 24; 22, 34 and 46 metaphases. 2 to 5 experiments at each MF. HEL, NCI-H460 and COL 320DM assays used 21 % oxygen, rather than anoxic conditions, as some anoxic karyotype modes are too close to 46.

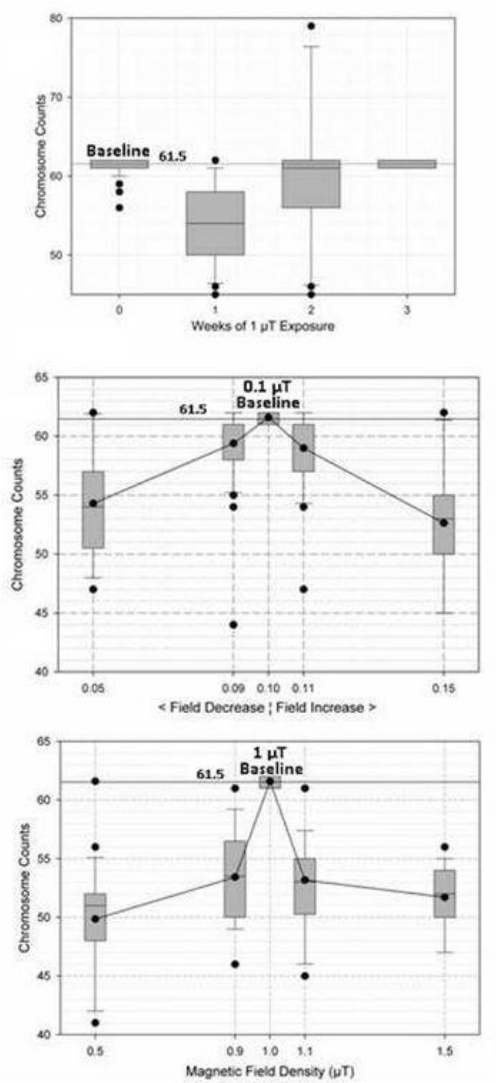


Fig. 4. TOP: K562 chromosome numbers return to baseline after 3 weeks of continuous 1 µT MF exposure. 65, 102, 50 and 37 metaphases. 2 experiments at each MF. MIDDLE: K562 Chromosome numbers obtained after 6 days by altering baseline MFs of 0.1 µT. 20, 31, 37 (baseline), 31, 35 metaphases. 3 to 6 experiments at each MF.
BOTTOM: For 1 µT, 28, 28, 37 (baseline), 28, and 28 metaphases. 3 experiments at each MF. Although the symmetry of the chromosome numbers is strong, there is more cell decay with increased than with reduced fields.

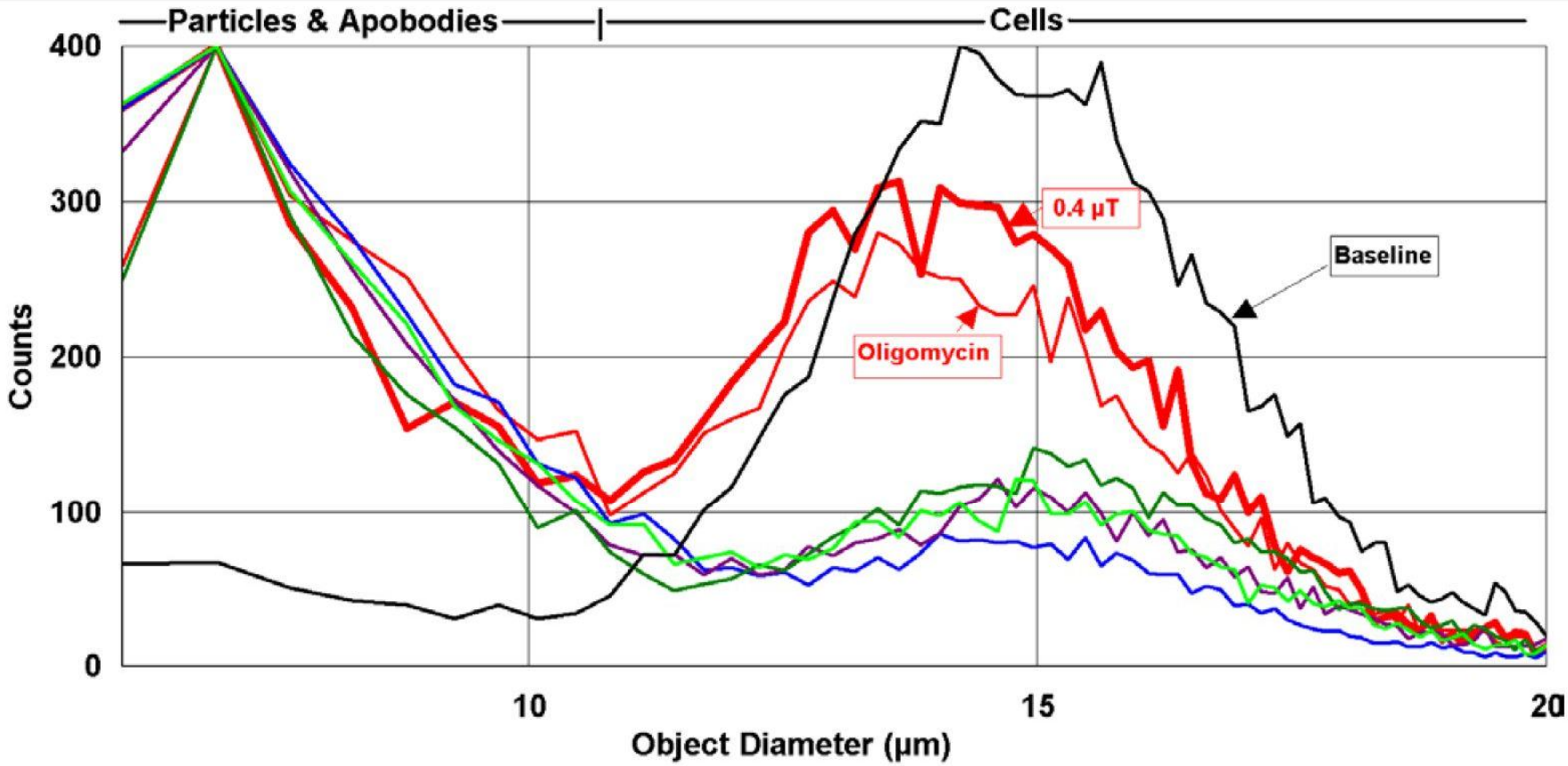
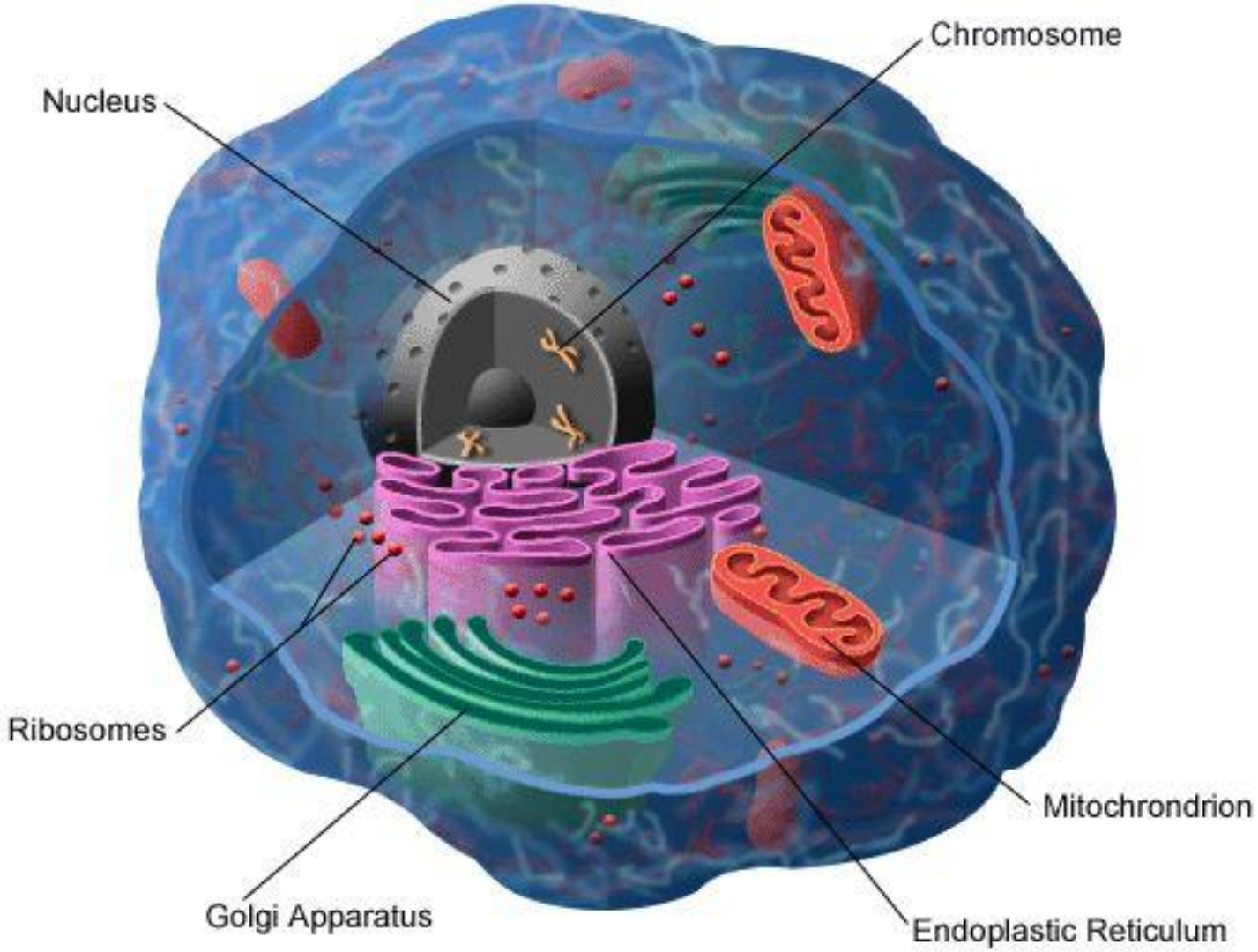


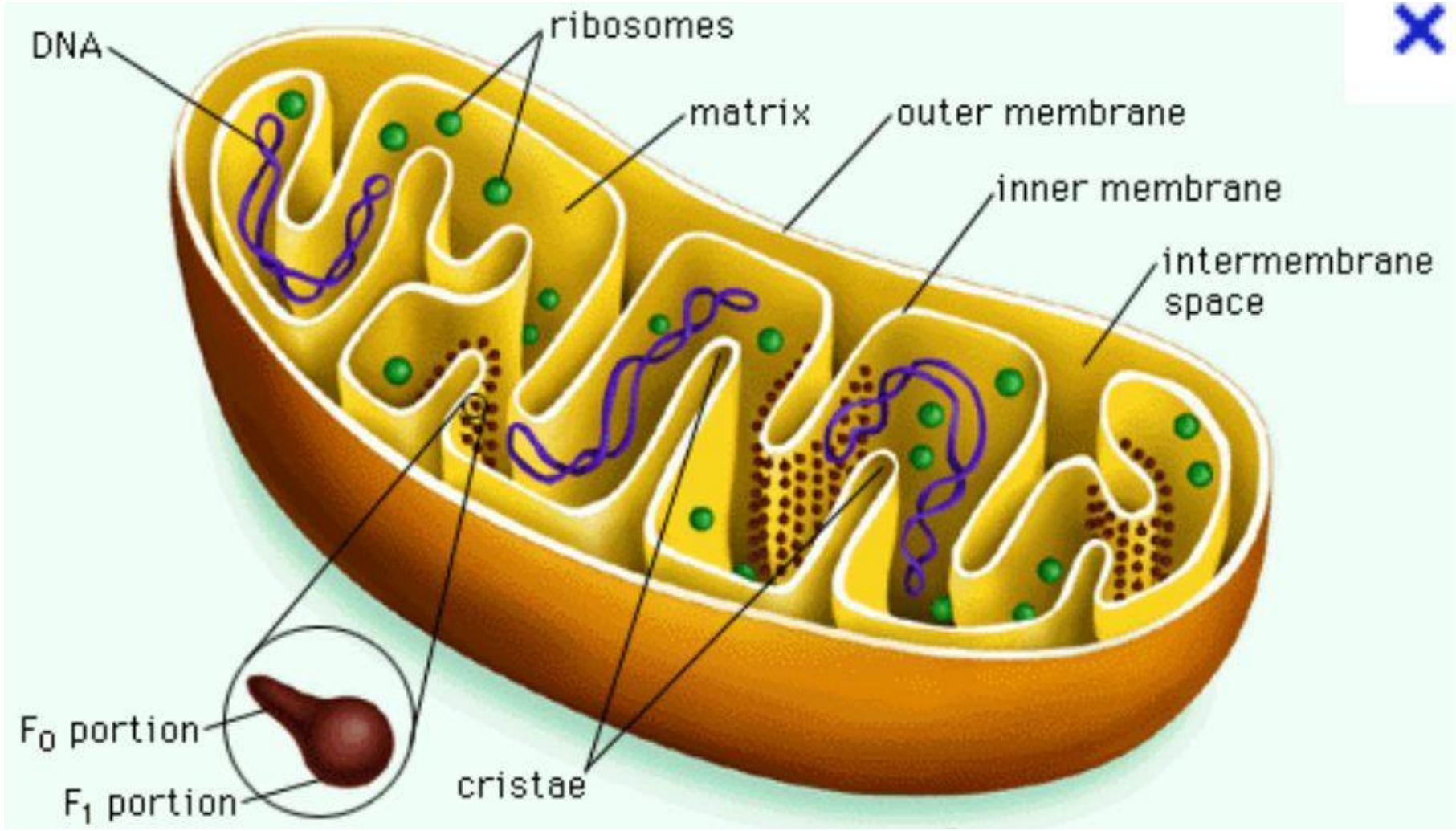
Fig. 3.18: Object Diameter histograms for 6-day anoxic exposures of K562 cultures to 0.4 µT MF at 60-Hz and oligomycin at 2.5 ng/ml. The lower 4 curves are: imatinib (0.04 µg/ml) in blue, resistin (40 ng/ml) in violet, metformin (0.01 mg/ml) in light green and melatonin-vitamin C (0.3 µg/ml, 26 µg/ml) in dark green. Exposed cultures are adjusted to a common small particle count maximum.

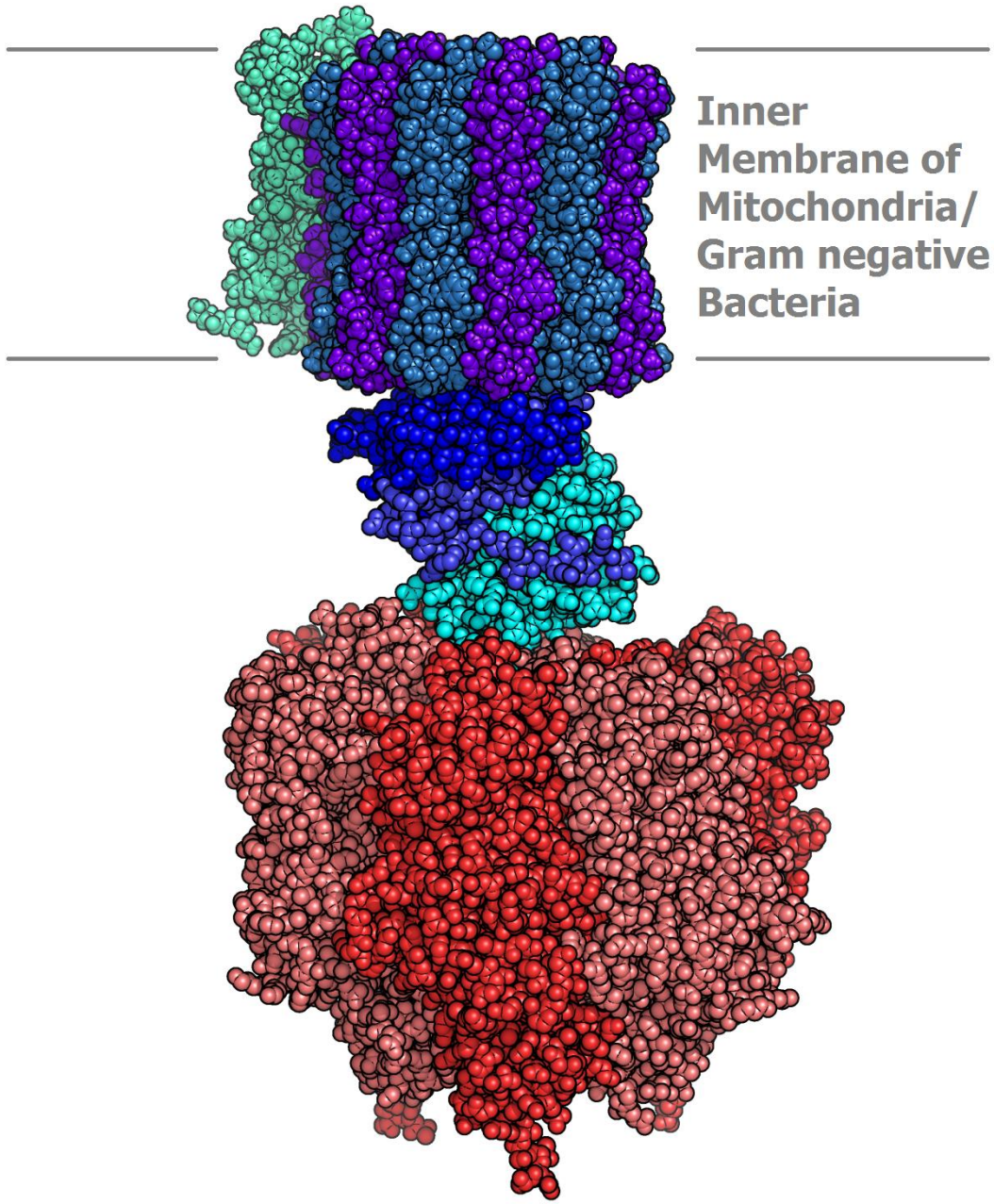
Site of Action

Site of Action

Anatomy of a Cell

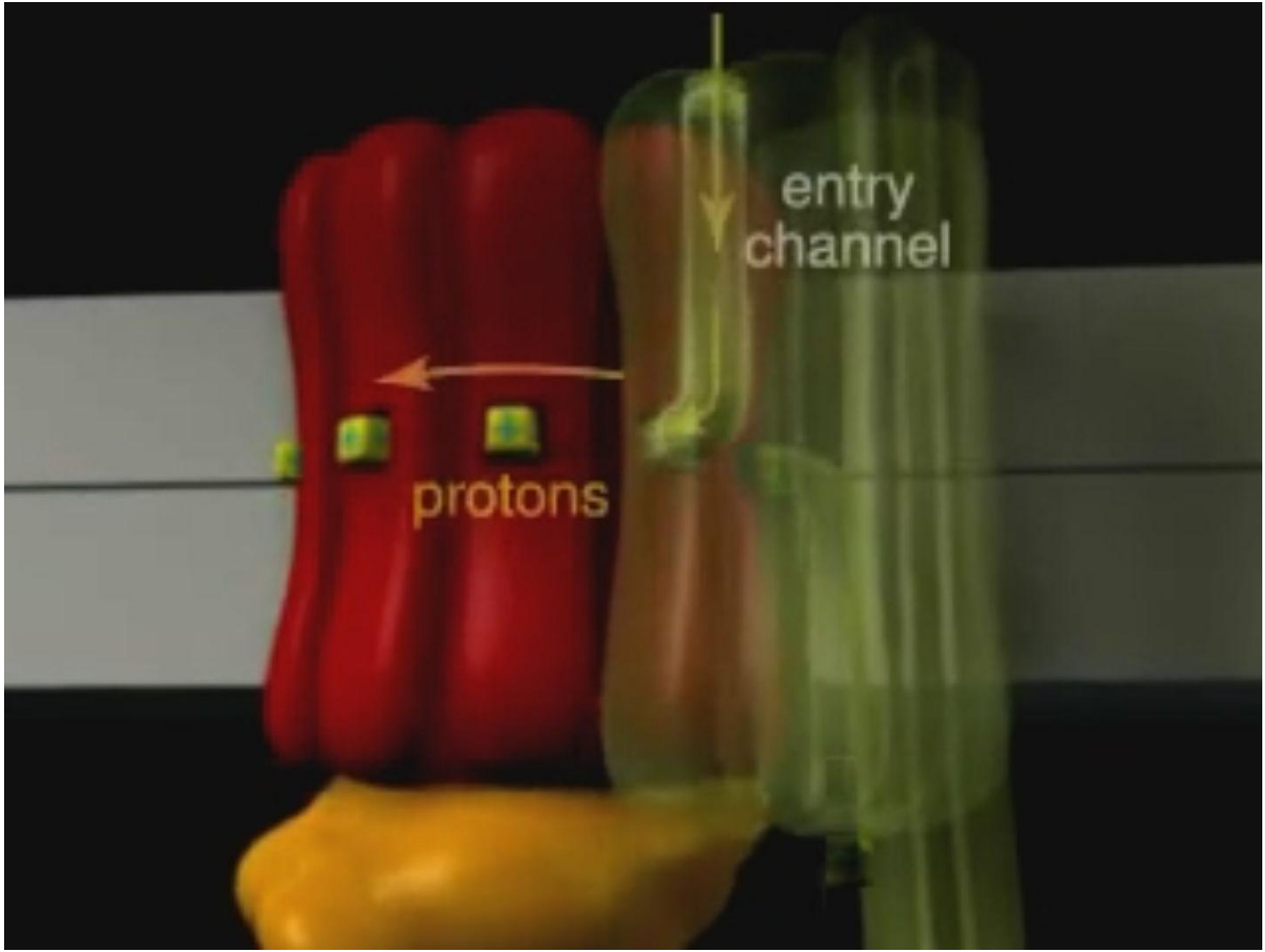


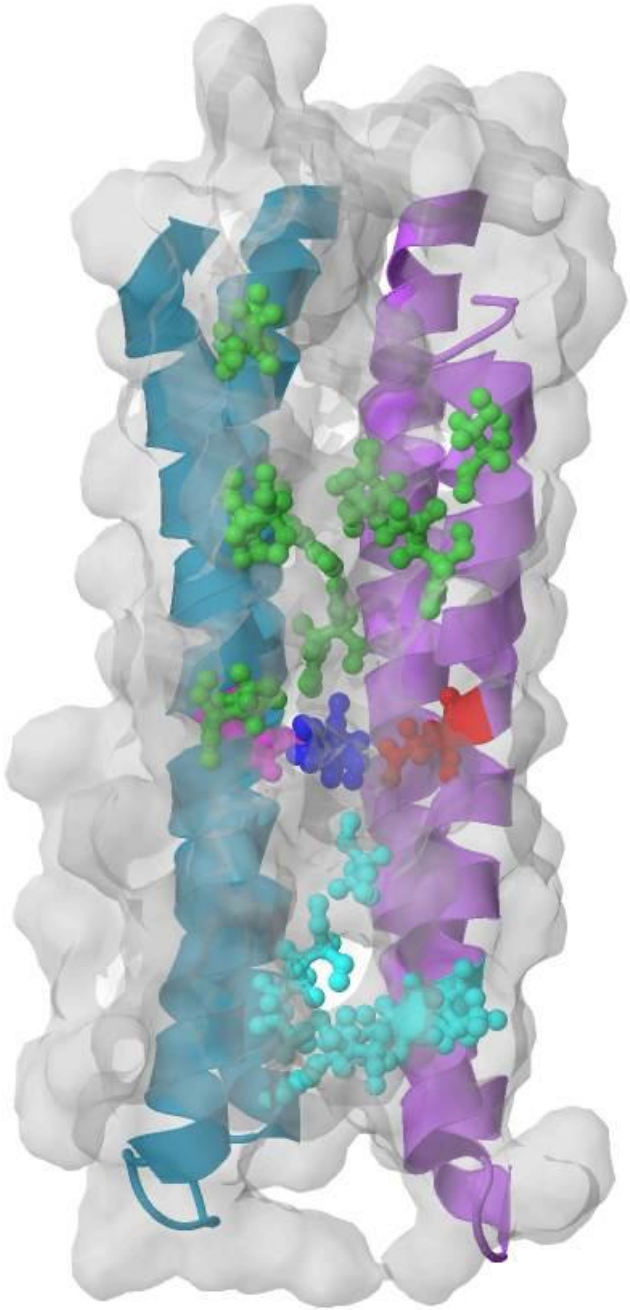

DNA
ribosomes
matrix
outer membrane
inner membrane
intermembrane space
F_0 portion
F_1 portion
cristae



ATP SYNTHASE

Water Channels


entry
channel
protons



Semikhina and Kiselev (1981 and 1988)

Lomonosov U, Moscow

Report alterations in the properties of water to which small ac magnetic fields are applied.

Magnetic fields change tg δ (ωRC) of water and the optical properties of dilute solutions of rhodamine 6G (a fluorescent molecule) over a wide range of field amplitudes: 25 nT to 879 μT.

"From all that has been said above, it follows that the higher the concentration of hydrogen bonds and proton-containing groups in aqueous systems, the greater will be the effects of weak fields on the aqueous systems."

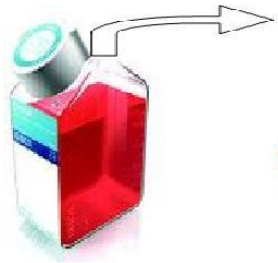
Reiter et al., 2011, Physics, U. of Houston

(arXiv:1101.4994v1 [cond-mat.mes-hall] 26 Jan 2011)

The tunnelling of protons exploited by ATPS has also been observed as double wells in neutron Compton scattering studies performed on water-filled nanotubes (scales of 2 nm or less). ATPS's water channels are contained within the 7.5 nm (diameter) rotor. Electrons in a scanning tunnelling microscope flow in a channel 0.5 nm in diameter.

Water Memory

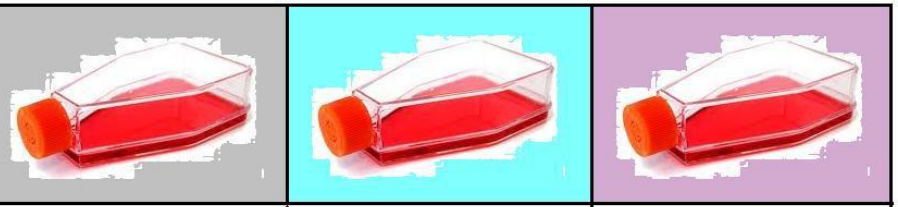
MEDIUM CONDITIONING



Cell Culture Medium is pipetted into 3 air-tight flasks

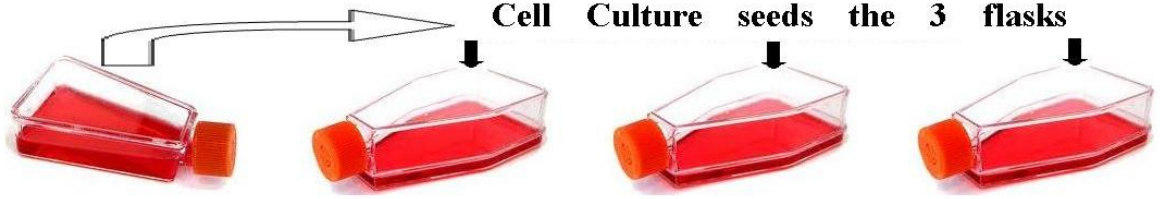
1

Medium only is exposed to different MFs for 15 h



Very Small MF	Incubator MF	Inhibitory MF

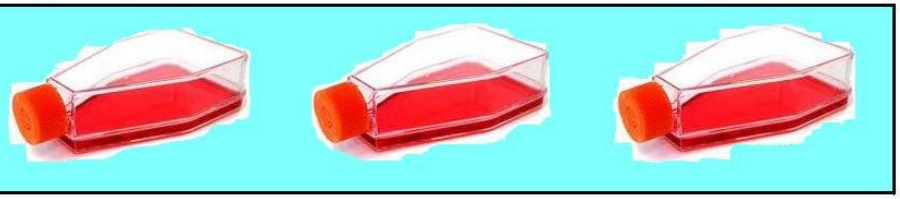
2



Cell Culture seeds the 3 flasks

3

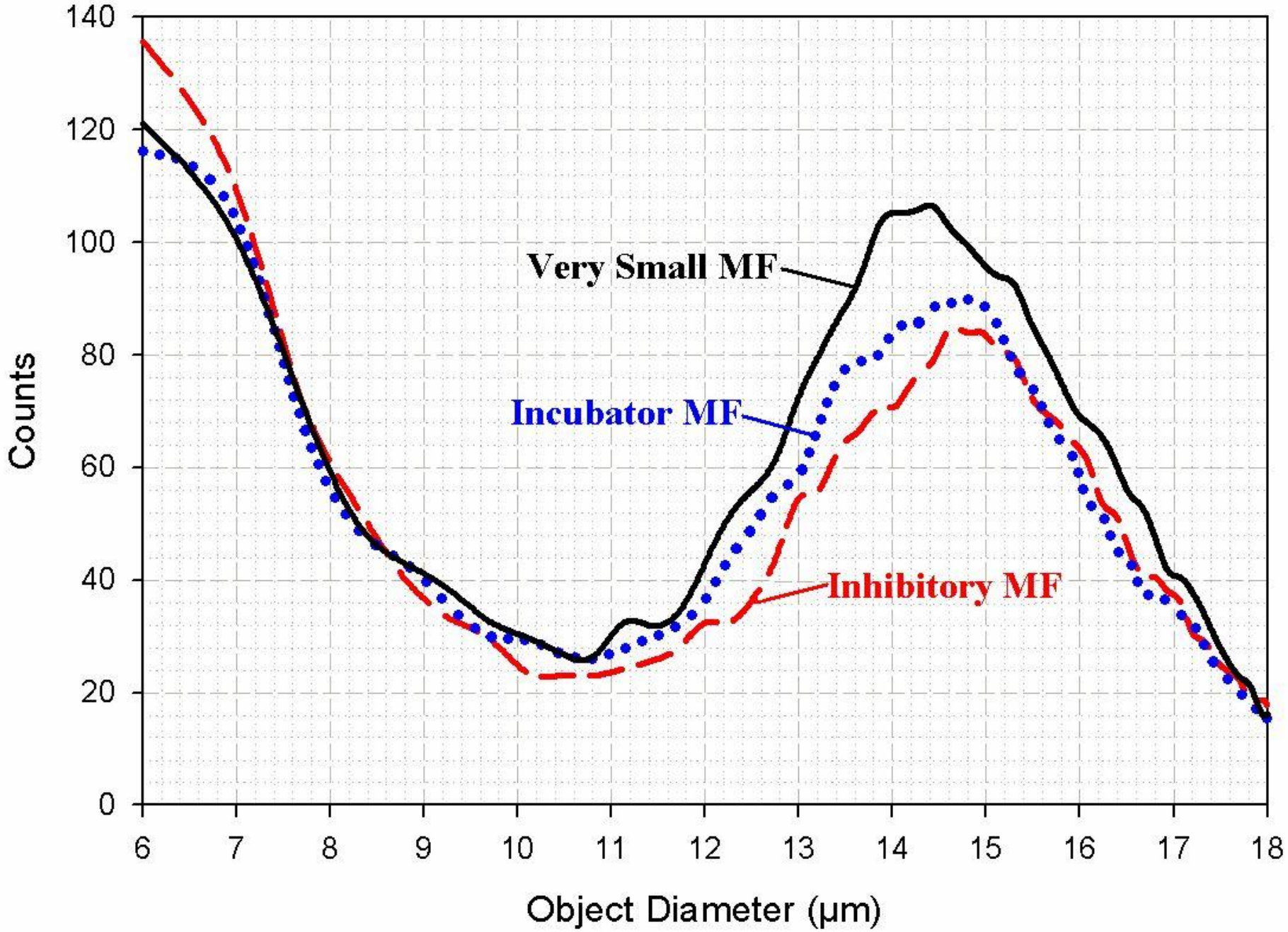
All flasks are incubated at Incubator MF for 7 h



4

Each flask is assessed with Scepter at 0 and 7 hours.

5


140
120
100
80
60
40
20
0
Counts
6
7
8
9
10
11
12
13
14
15
16
17
18
Object Diameter (µm)
Very Small MF
Incubator MF
Inhibitory MF

Predictions of Semikhina and Kiselev on water alterations confirmed in biological measurements

1. MF effects on chromosome numbers are observed starting at the threshold (25 nT) predicted, and extending over two orders of magnitude.

2. Resonances at specific frequency-amplitude combinations were confirmed.

3. Removing the static MF acted on water variables in a direction opposite to the application of ELF MFs, as predicted. Thus, elimination of ELF and static MFs allows water to optimize its molecular arrangement, which improves ATPS efficiency.

4. Effects of ELF MFs can be carried by medium alone.

5. Effects of ELF MFs are detectable in small pH changes.

Proofs based on biological measurements

1. 5 different cancer cell lines representative of the most common human cancers display a reduction in their chromosome numbers when exposed to 60-Hz MFs.

2. The cell size histogram measured for MFs uniquely matches the characteristic of the specific ATPS Fo inhibitor oligomycin A (3.2 nM).

3. Observations imply a disruption in water structure with little sensitivity to MF intensity, or to the particularities of specific cell type metabolism: the knockout of an important biological enzyme by changes in water structure.

4. If MFs inhibit ATPS, they should also activate AMPK. This was confirmed using AMPK inhibitors (resistin) and activators (metformin).

The "kT problem":

MF energies are smaller than thermal energies, and are overwhelmed

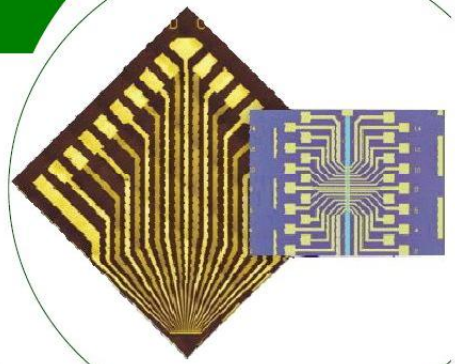
$$Most\ Probable\ Speed = \left(\frac{2\ kT}{m}\right)^{1/2}$$

(Maxwell's Distribution)

Tunneling

SpinTJ™

Magnetic Field Sensors



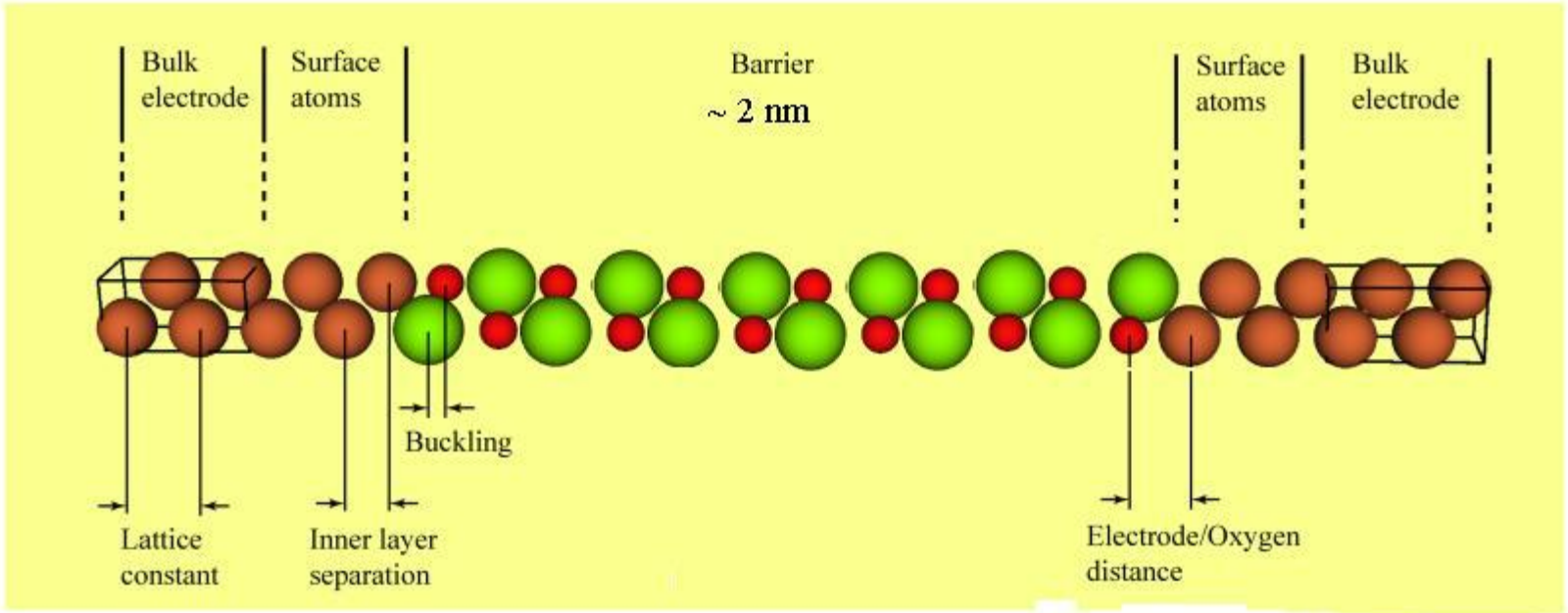
Micro Magnetics
Sensible Solutions

General Purpose Low Noise Sensor:

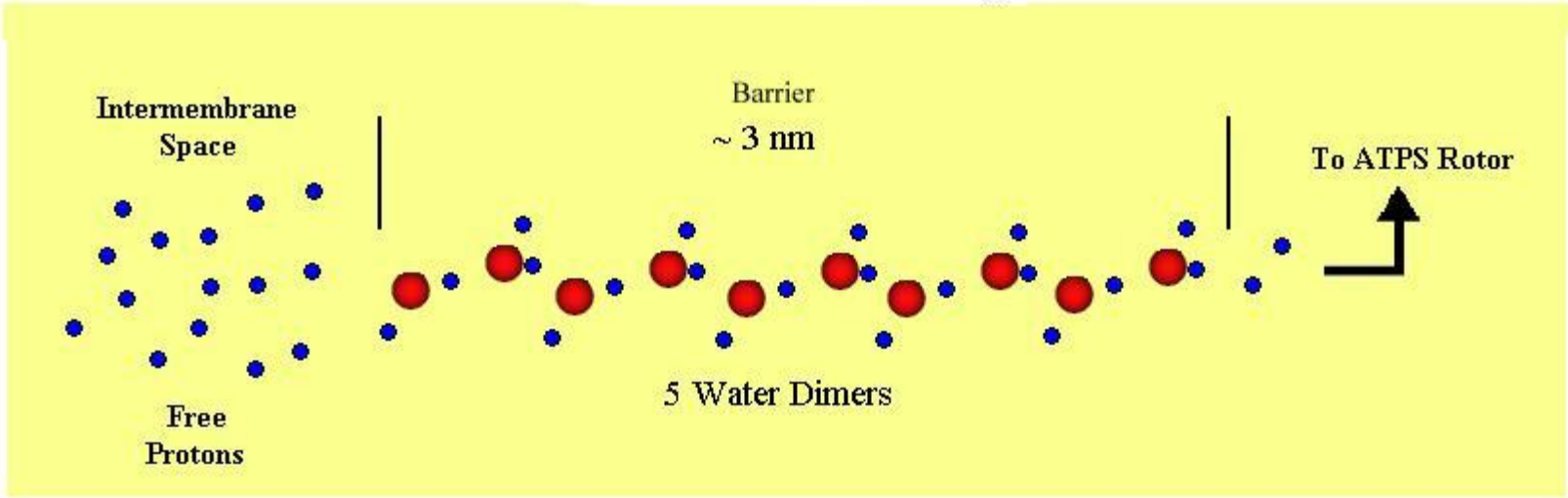
- 0.9 mm spatial resolution

- detectability: 0.26 nT at 60-Hz

Semiconductor Tunnelling



ATPS Tunnelling



Some tunnelling solutions are known for electrons

In the case where there is no magnetic field, tunnelling through the water string would be adiabatic and the rate of transfer would be

$$w = \left\{\frac{2\pi}{\hbar}|M|^2\delta\left(E_\psi - E_\chi\right)\right\}^5$$

where M is the tunnel matrix obtained from the overlap of the solution of Schrödinger equation for both sides of the tunnel (ψ and χ).

When a magnetic field of frequency ω is applied, the original Hamiltonian is perturbed and the transition is into states that differ by energy $\hbar\omega$ from the energy of the initial state (Fermi's golden rule).

$\hbar\omega =$ 2.48 x 10^{-13} eV at 60 Hz

Conclusions

Magnetic fields are strongly bioactive, suppressing metabolism at a time scale of hours, through an effect on water based in Quantum Electrodynamics. The effect is not acutely evident because of long time constants and because of tight ATP level regulation by AMPK.

On the basis of physiological evidence *in vitro* only, Environmental Magnetic Field exposures may

- **have had an effect on general cancer rates in exposed populations (some epidemiological evidence was compiled by IARC for leukemia),**
- **have influenced the incidence of diabetes in populations,**
- **have increased the overall longevity of populations.**