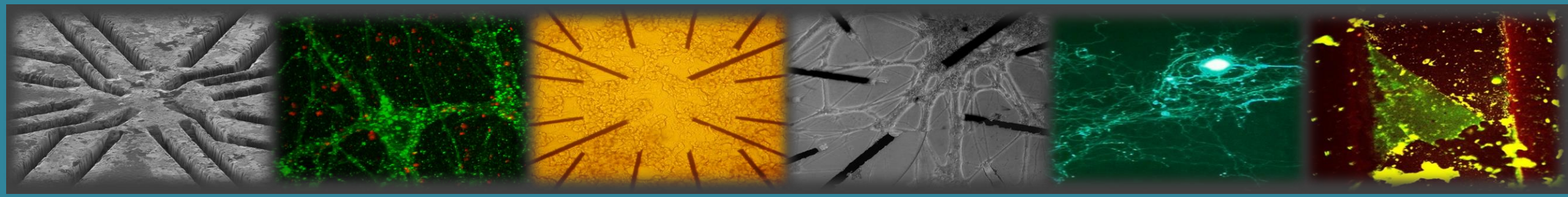
UNIVERSITA' DEGLI STUDI DI TORINO

Solid State Physics group

Fisica Torino PHYSICS DEPARTMENT

Diamante artificiale: applicazioni nella bio-sensoristica



http://www.ph.unito.it/dfs/solid/index.html

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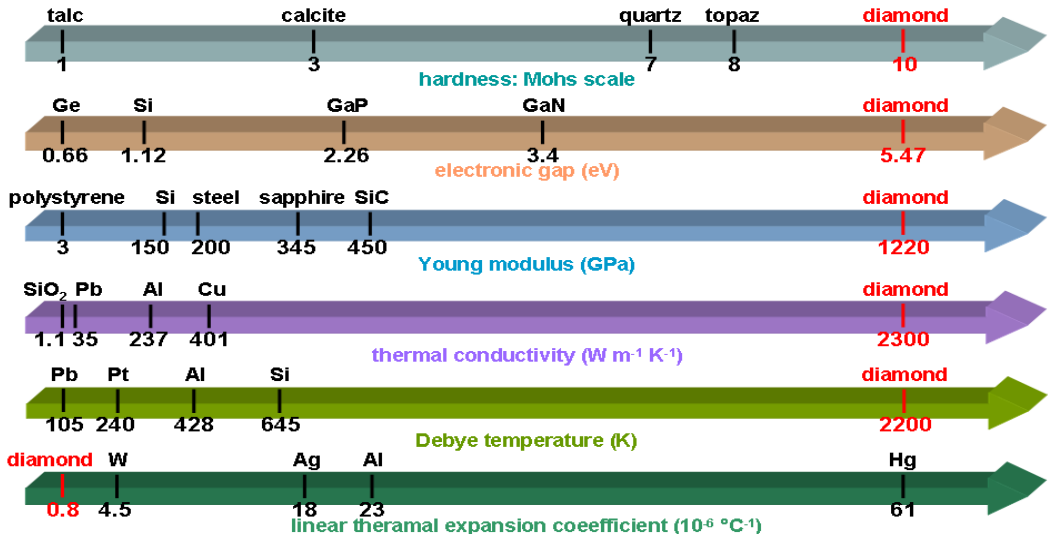
INFN Istituto Nazionale di Fisica Nucleare
SEZIONE DI TORINO

MeV ION BEAM LITHOGRAPHY

Diamond properties

Cellular bio-sensor

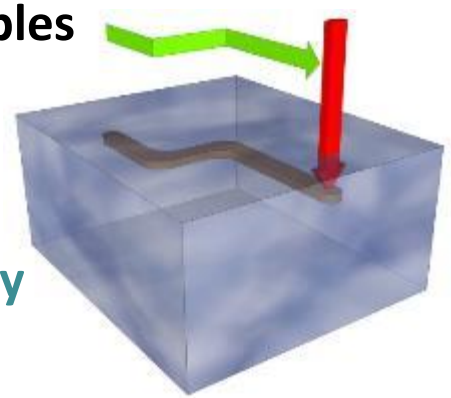
- bio-compatibility
- chemical inertness
- optical transparency



Ionizing radiation detector

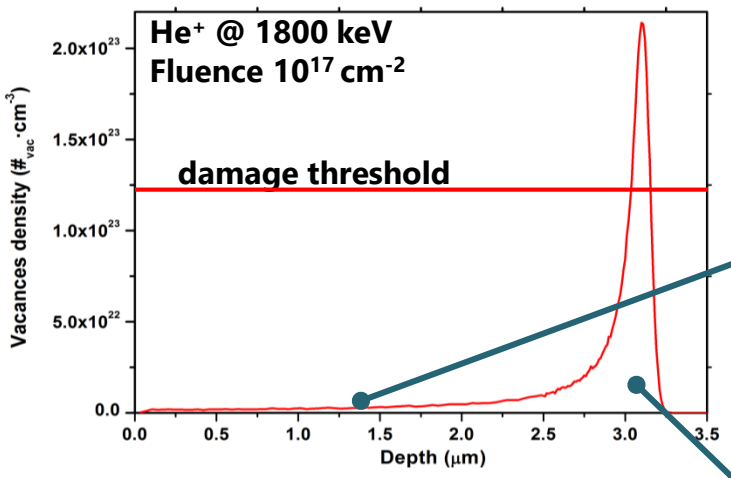
- Radiation hardness
- Tissue equivalence
- High carrier mobility
- High breakdown field

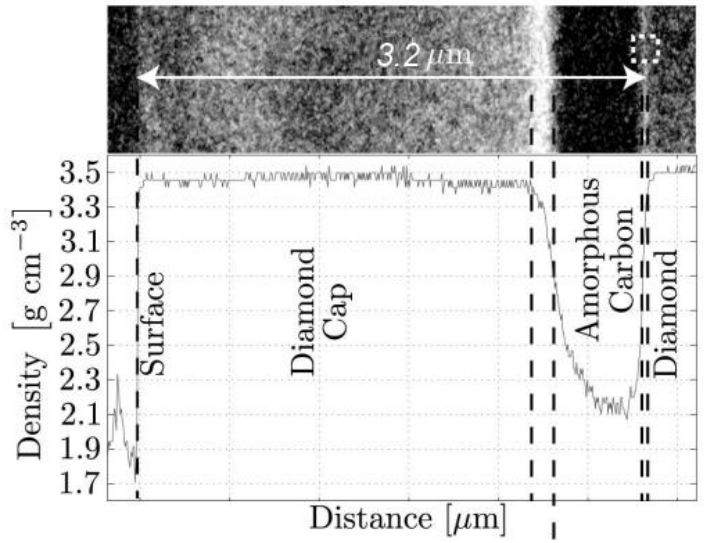
- diamond synthesis: a mature technology: availability of synthetic monocrystalline samples of high quality (electronic grade)

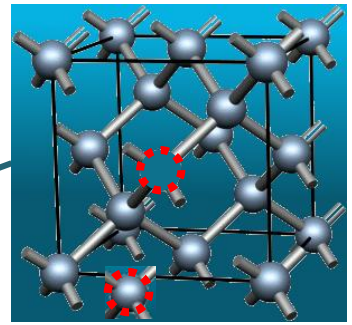


- diamond fabrication: Ion Beam Lithography

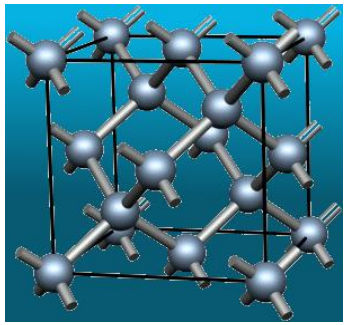
MeV ion induced damage in diamond





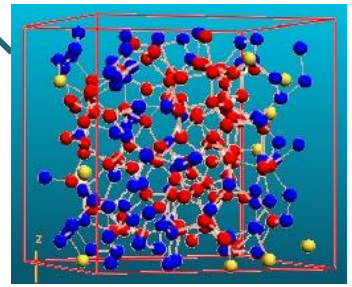


Thermal annealing

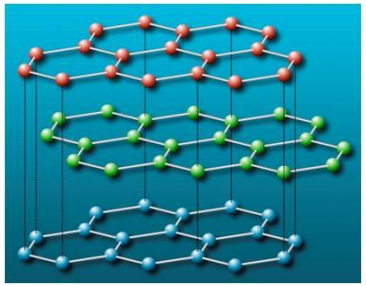


Below threshold: diamond with Frenkel defects → **diamond**

High fluence implantation → formation of an amorphous carbon layer where the damage density exceeds a threshold



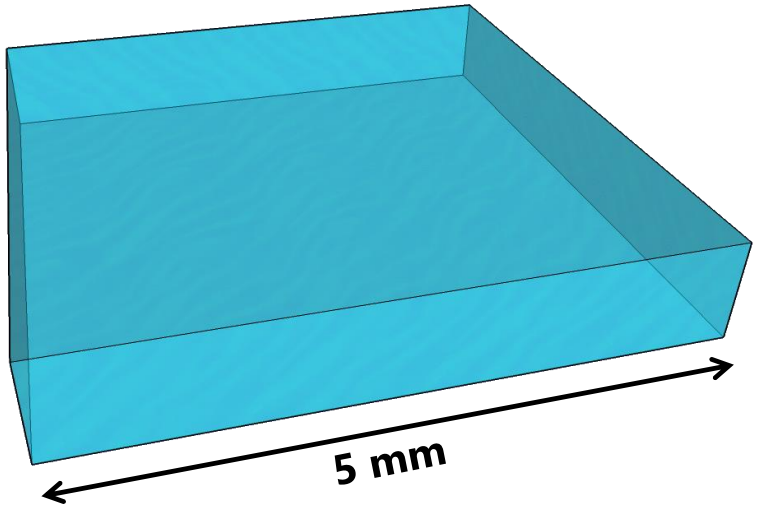
Thermal annealing



Above threshold: amorphous carbon → **nanocrystalline graphite**

A. Silverman et al., **Physical Review B** 83, 224206 (2011)
B. A. Fairchild et al., **Advanced Materials** 24, 2024 (2012)

MeV collimated ion beam lithography



Direct fabrication of graphitic electrodes into diamond crystal

Parallel fabrication

Sensor dimensions: up to 20 mm^2

Electrodes resolution: 100 – 300 nm

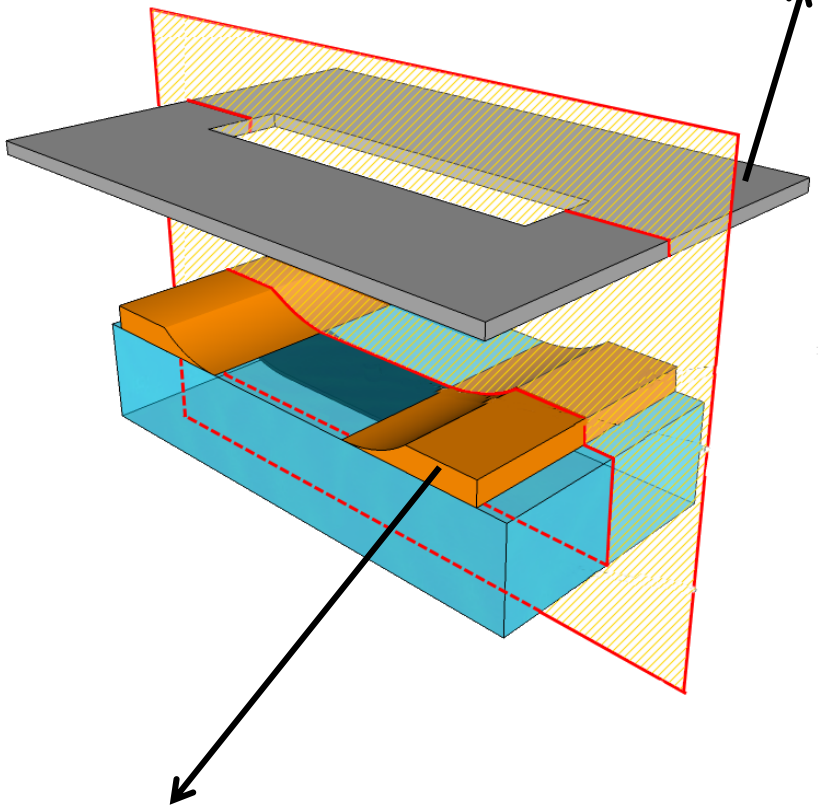
High power laser or Focused Ion Beam **micro/nano machined mask** for broad MeV ion beam implantation

- Variable thickness mask -

"**Fabrication and electrical characterization of three-dimensional graphitic microchannels in single crystal diamond**", F. Picollo, et al., **New Journal of Physics 14 (2012) 053011**
"**All-carbon multi-electrode array for real-time in vitro measurements of oxidizable neurotransmitters**", F. Picollo, et al., **Scientific Reports 6, (2016) 20682**

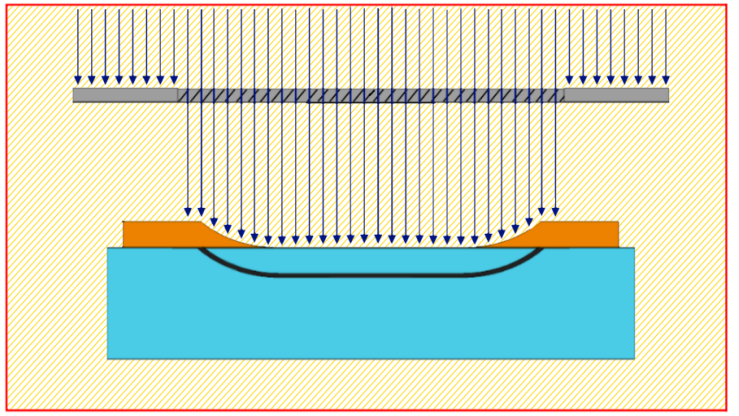
MeV collimated ion beam lithography

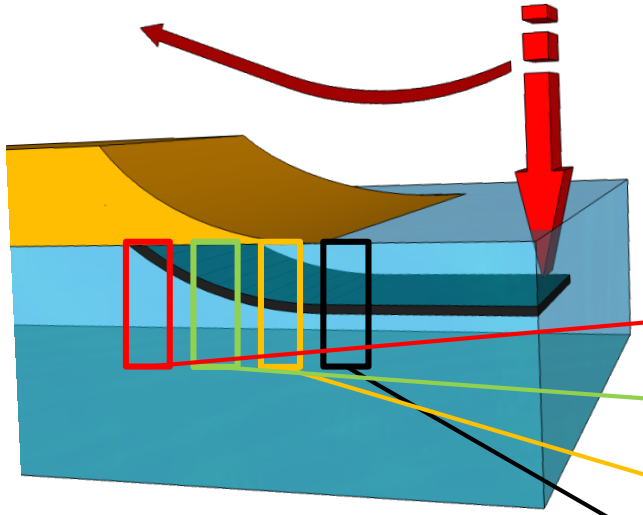
Two systems masks

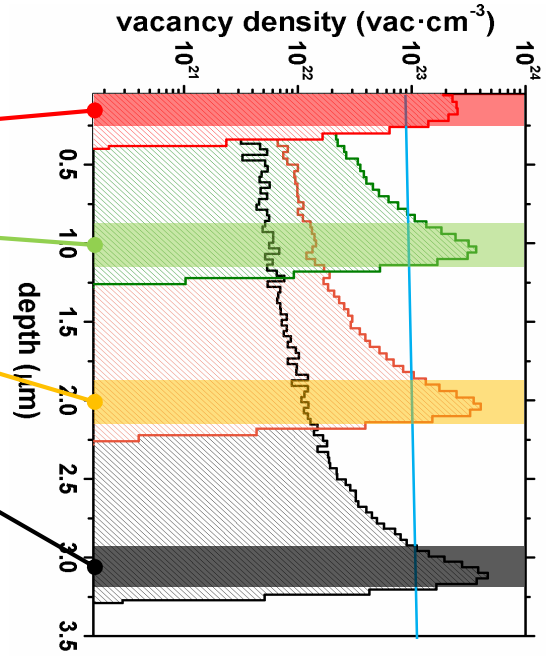


Freestanding mask - collimation

- laser microfabricated thin metal film (>5µm)
- definition of lateral geometry of electrodes



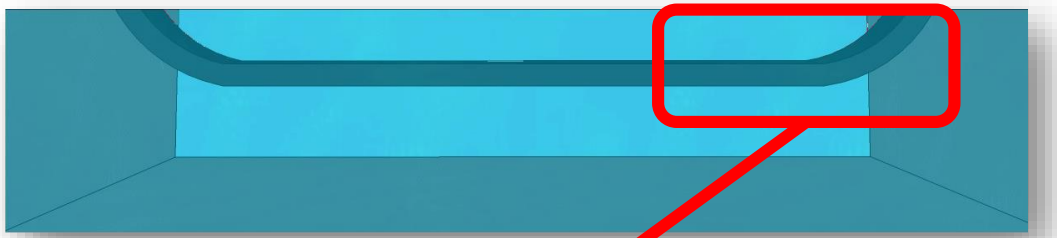


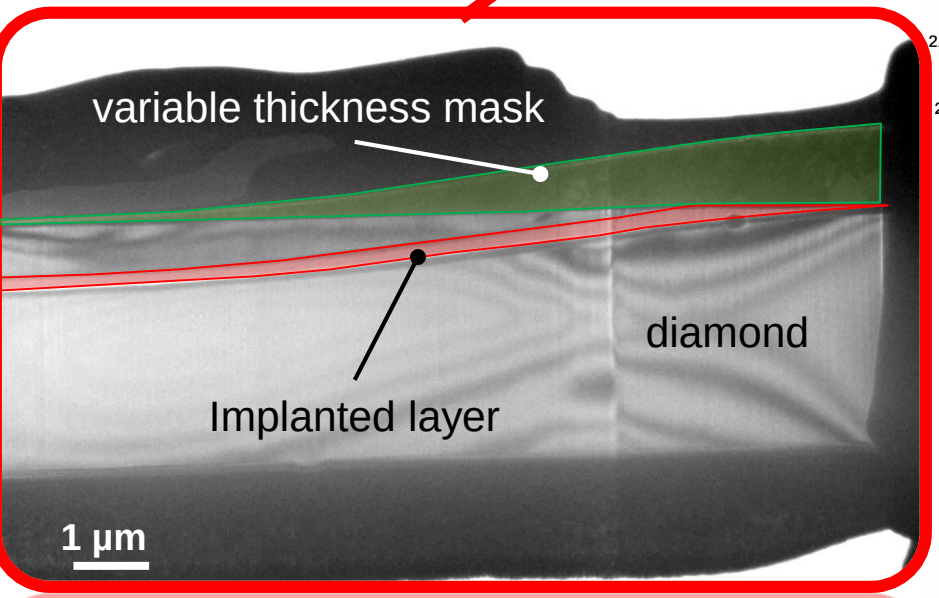


Variable thickness mask – depth modulation

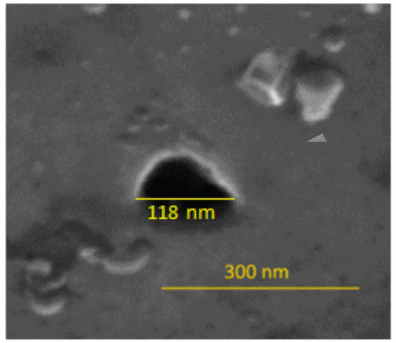
- Deposition of metal over diamond surface (>5µm)
- Control of ion penetration = depth of electrode

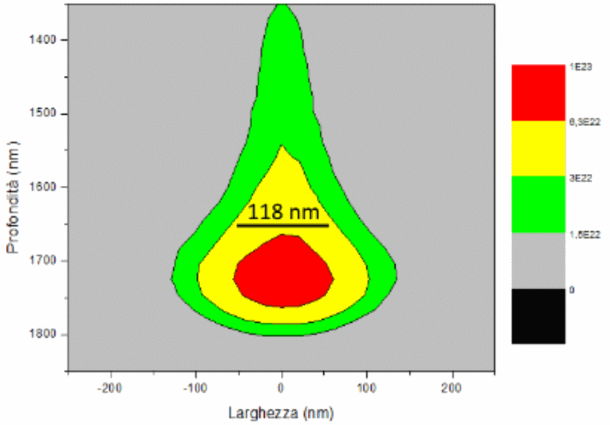
MeV collimated ion beam lithography

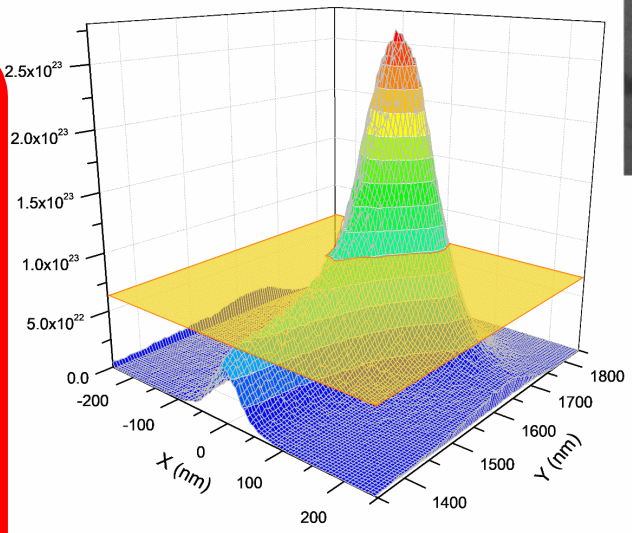


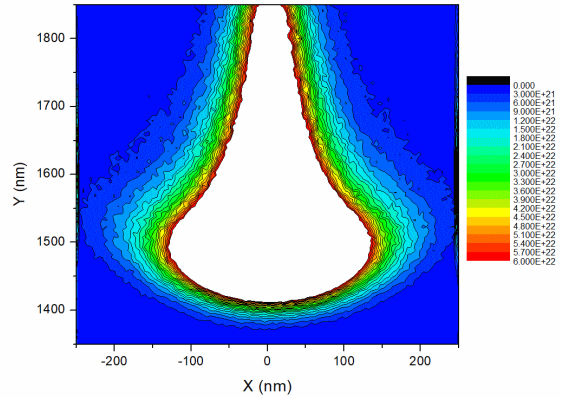


Cross-section of the channel







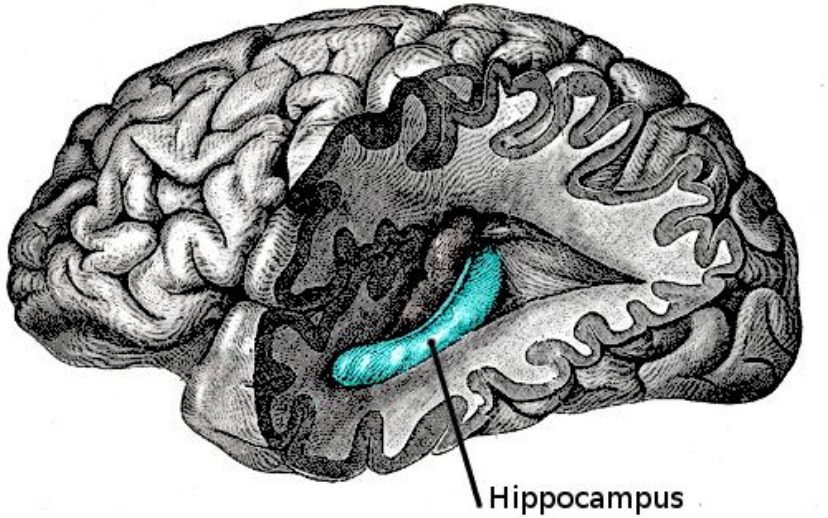


SINGLE CRYSTAL DIAMOND BIOSENSORS

Neurodegenerative diseases

Alzheimer's disease

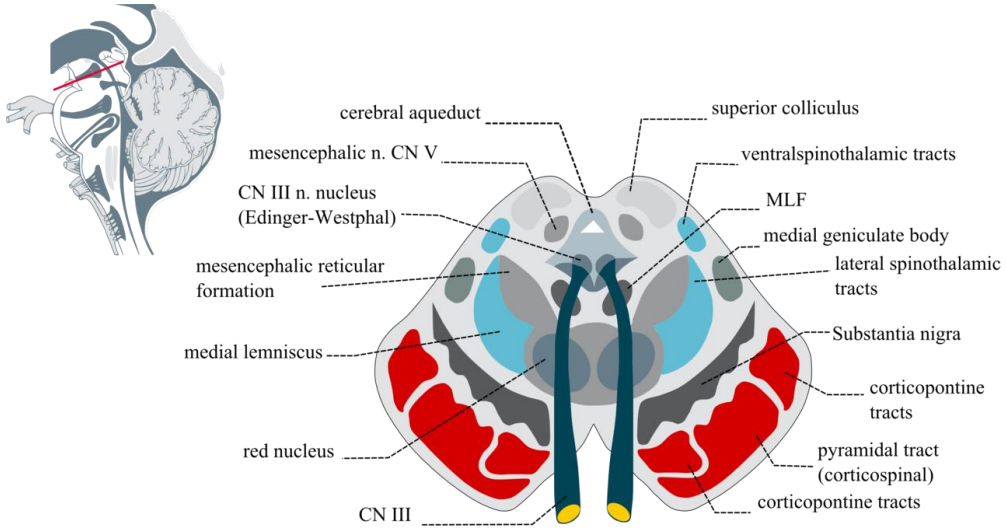
consequence of several cellular degenerative processes, primarily affecting memory encoding brain regions, such as hippocampus



By Henry Vandyke Carter - Henry Gray (1918) Anatomy of the Human Body (See "Book" section below)Bartleby.com: Gray's Anatomy, Plate 739, Public Domain, https://commons.wikimedia.org/w/index.php?curid=3907047

Parkinson disease

progressive degeneration of the *substantia nigra* pars compacta (SNc) dopaminergic neurons



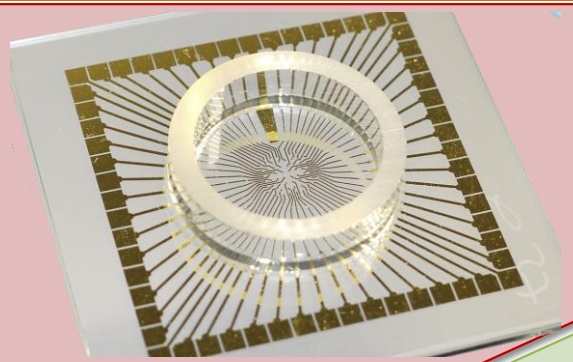
By Madhero88 - Own work, CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=7157181

Neurodegenerative diseases such as **Parkinson** and **Alzheimer's disease** (PD, AD) are characterized by a long lasting **asymptomatic phase** during which neurons alter their synaptic and excitable properties without clearly affecting brain function

Biosensing on excitable cells

Standard commercial detector

→ Multi electrode arrays (MEA) ←



Detection technique

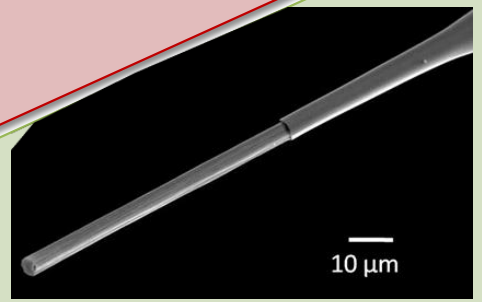
- Potentiometry

Drawback

- Only potentiometric measurement

ACTION POTENTIAL

EXOCYTOSIS



Standard commercial detector

→ Carbon fiber electrodes (CFE) ←

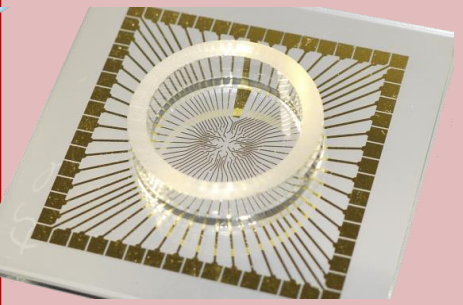
Detection technique

- Amperometry

Drawback

- One cell measure + only amperometric measurement

Biosensing on excitable cells



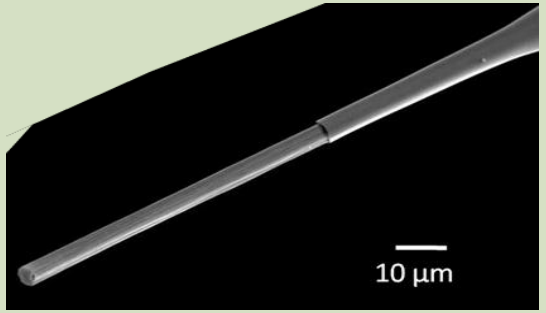
ACTION POTENTIAL

potentiometry

Multi technique
diamond biosensor

amperometry

EXOCYTOSIS



16 ch MEA: Amperometry or Potentiometry

diamonds:

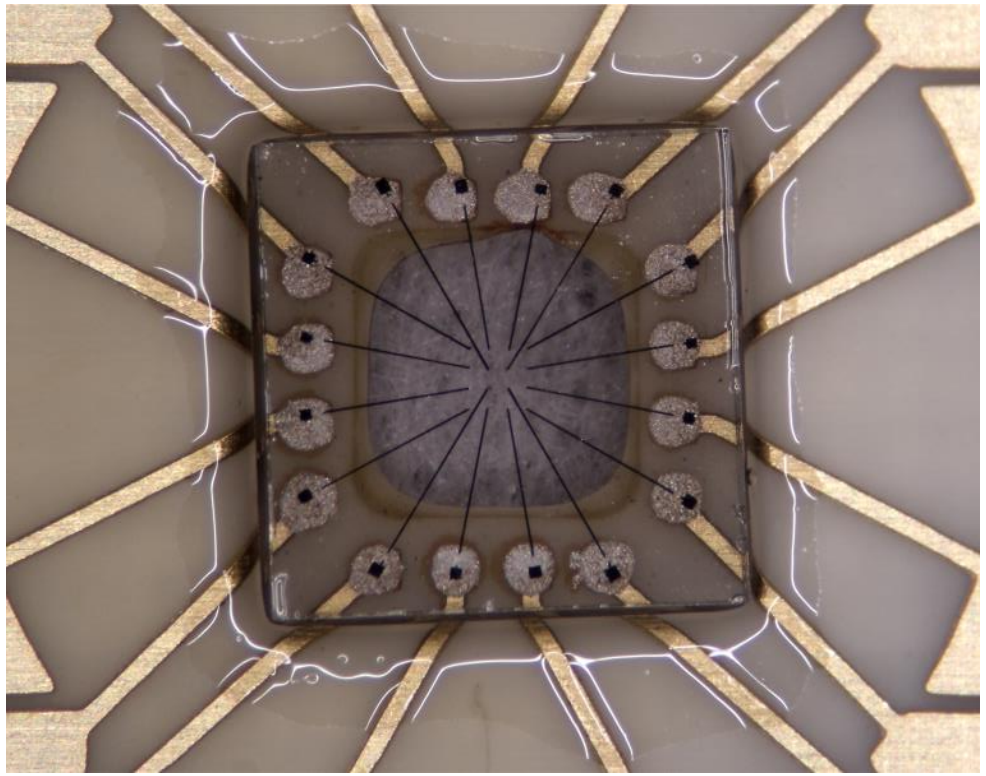
- Chemical Vapour Deposition
- single crystal
- type IIa
- 4.5×4.5×0.5 mm^3

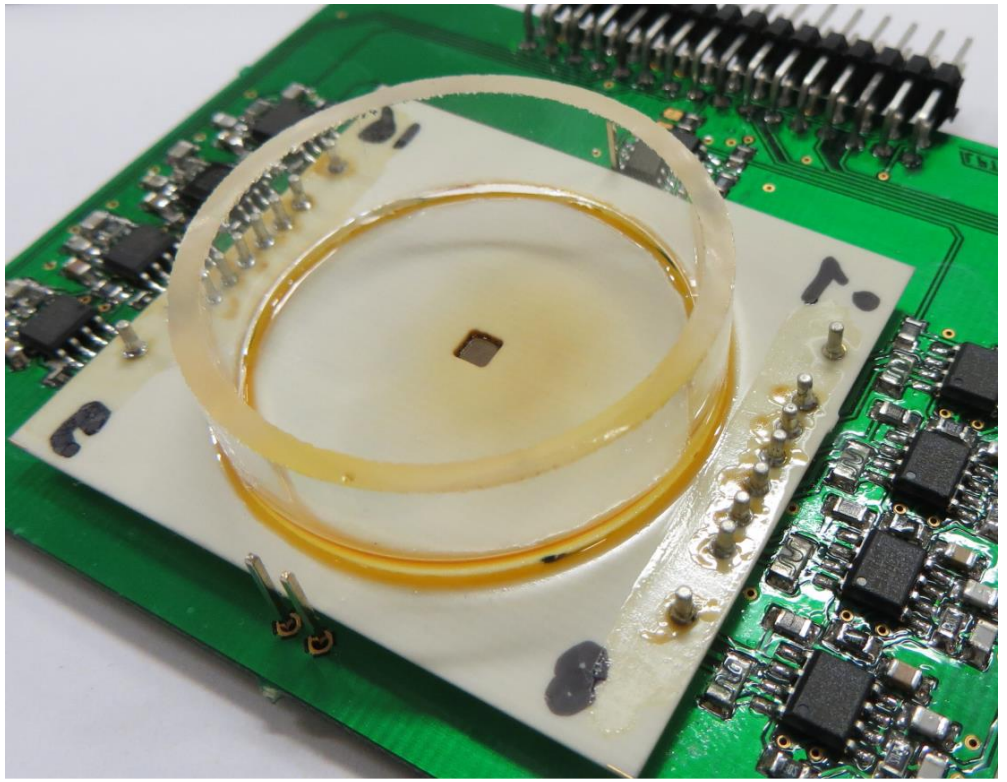
implantation:

- He^+ @ 1.2 MeV
- fluence $1.2 \cdot 10^{17}$ cm^{-2}
- penetration depth ~2 µm

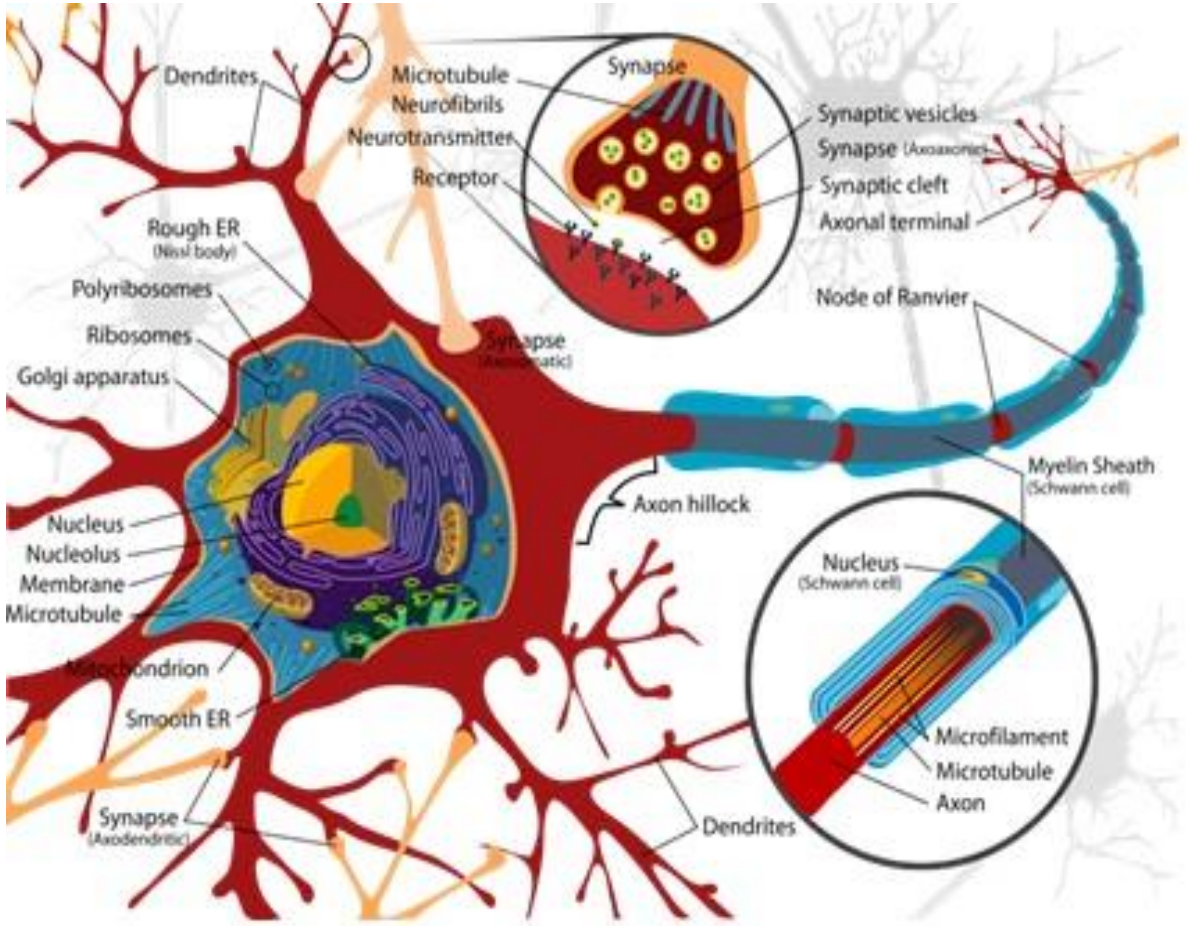
thermal treatment:

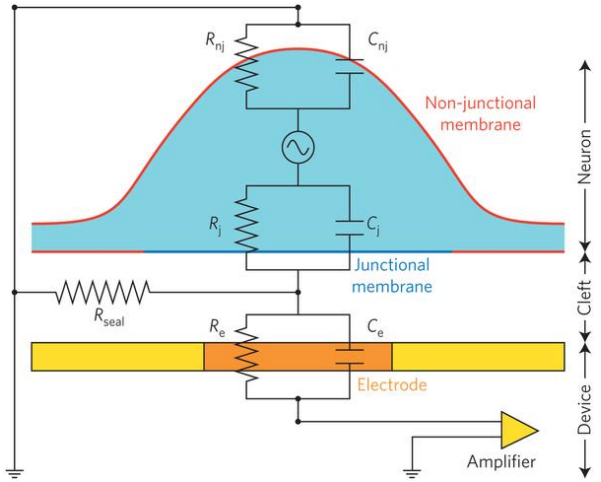
- 950 °C for 2 hours
- ~10^{-6} mbar



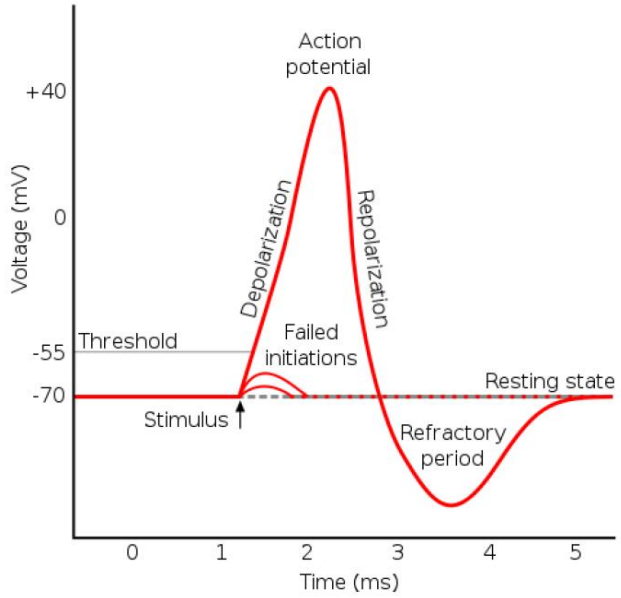


Potentiometric detection of Action Potential





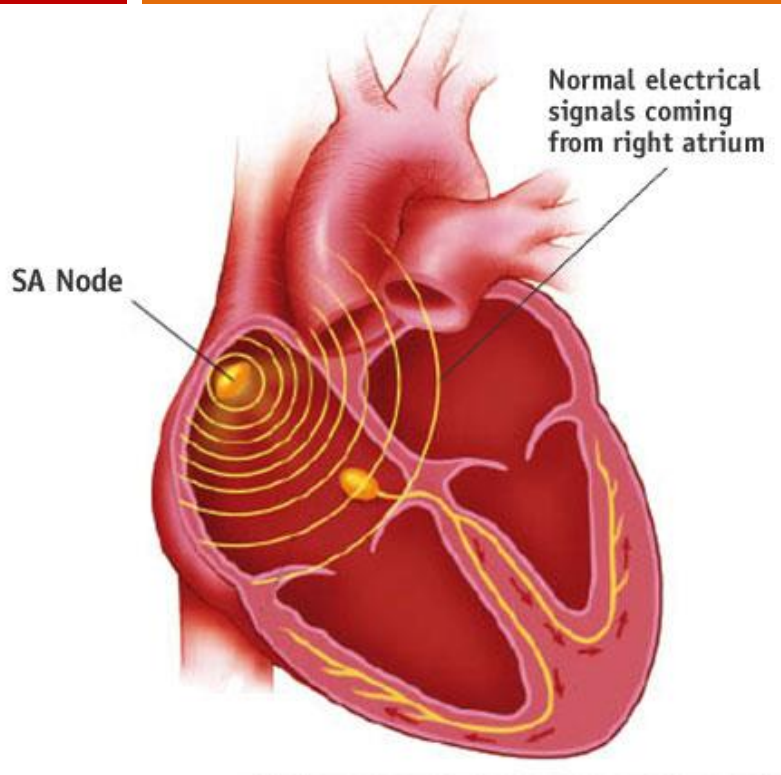
M.E. Spira & A Hai,
Nature Nanotechnology,
8, 83 (2013)



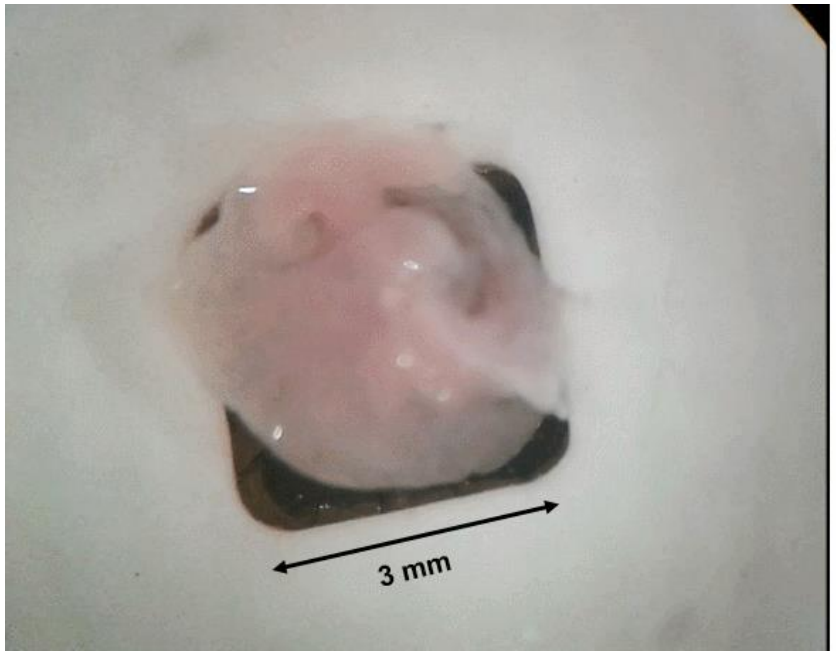
Action Potential

- due to sodium and calcium channels opening
- duration 1 - 100 ms
- membrane depolarizations from -65 mV to +45 mV

Sinoatrial node & potentiometry

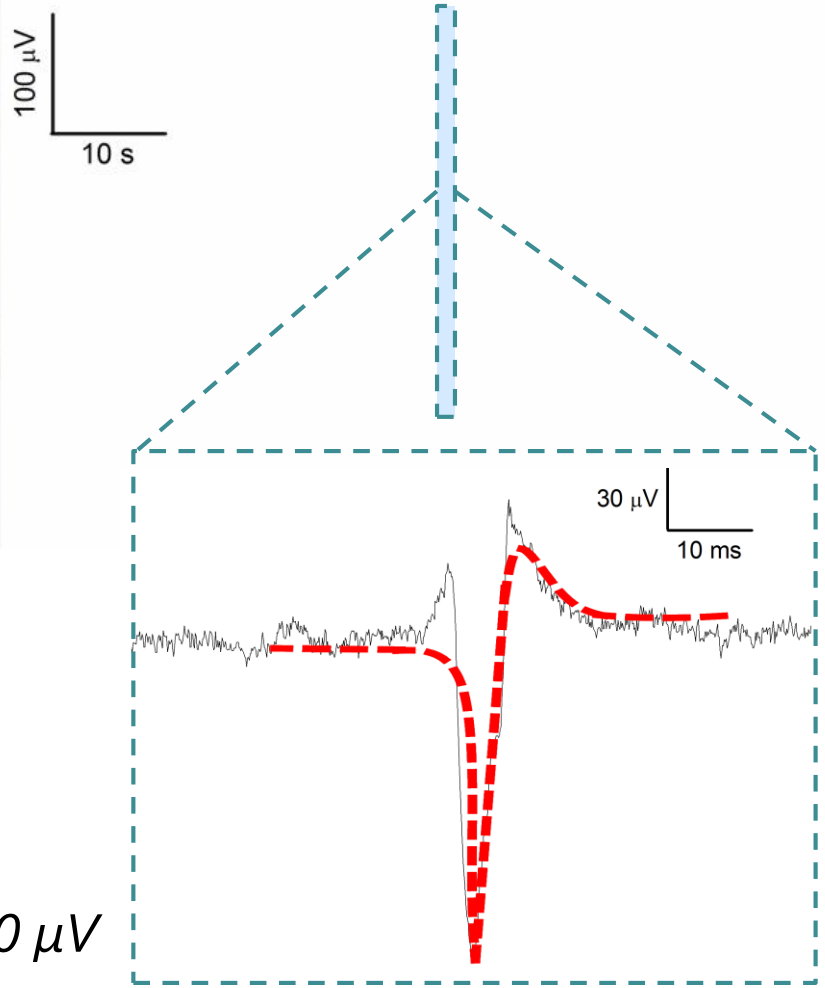


- natural ***pacemaker*** of the **heart**
- govern frequency and strength of atrial contraction

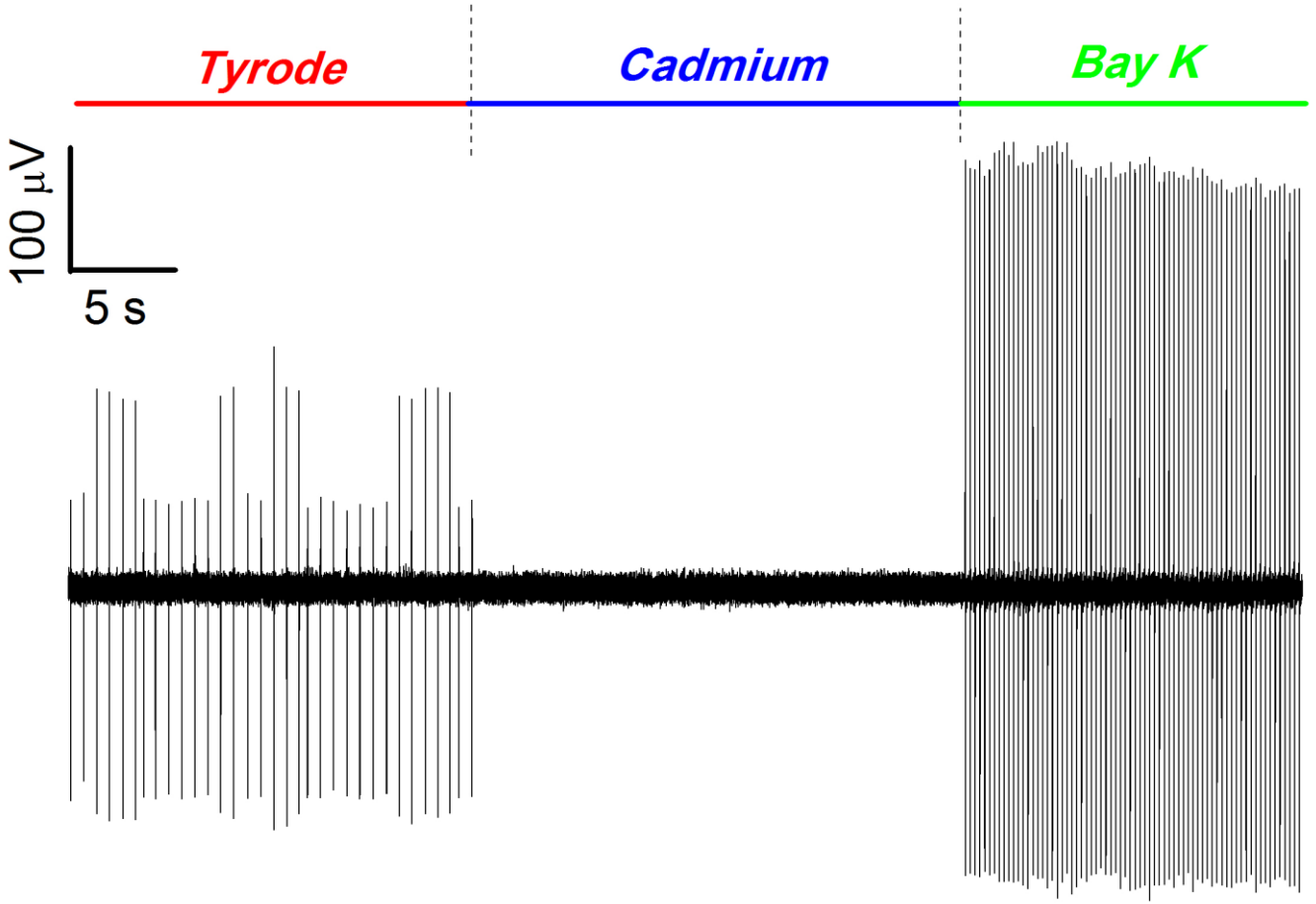


Sinoatrial node slice with heart muscular tissue residue

- Noise: *20 µV*
- *Spikes frequency*: *2 Hz*;
- Mean signal amplitude: *300 µV*



Potentiometric detection of action potential



Saline solution
(Tyrode)

f ~ 2 Hz
I ~ *300 µV*

Pharmacological experiment

Cadmium solution
500 µM
Calcium channels blocker

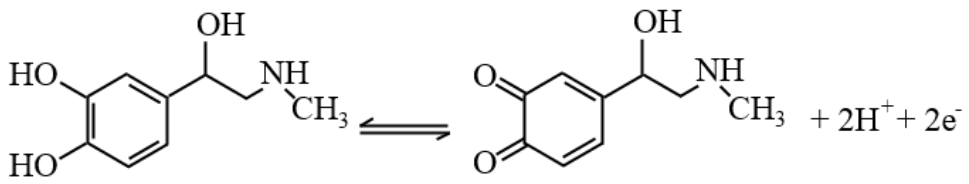
f ~ 0 Hz
I ~ *0 µV*

***Bay K* solution**
10 µM

f ~ 5 Hz
I ~ *600 µV*

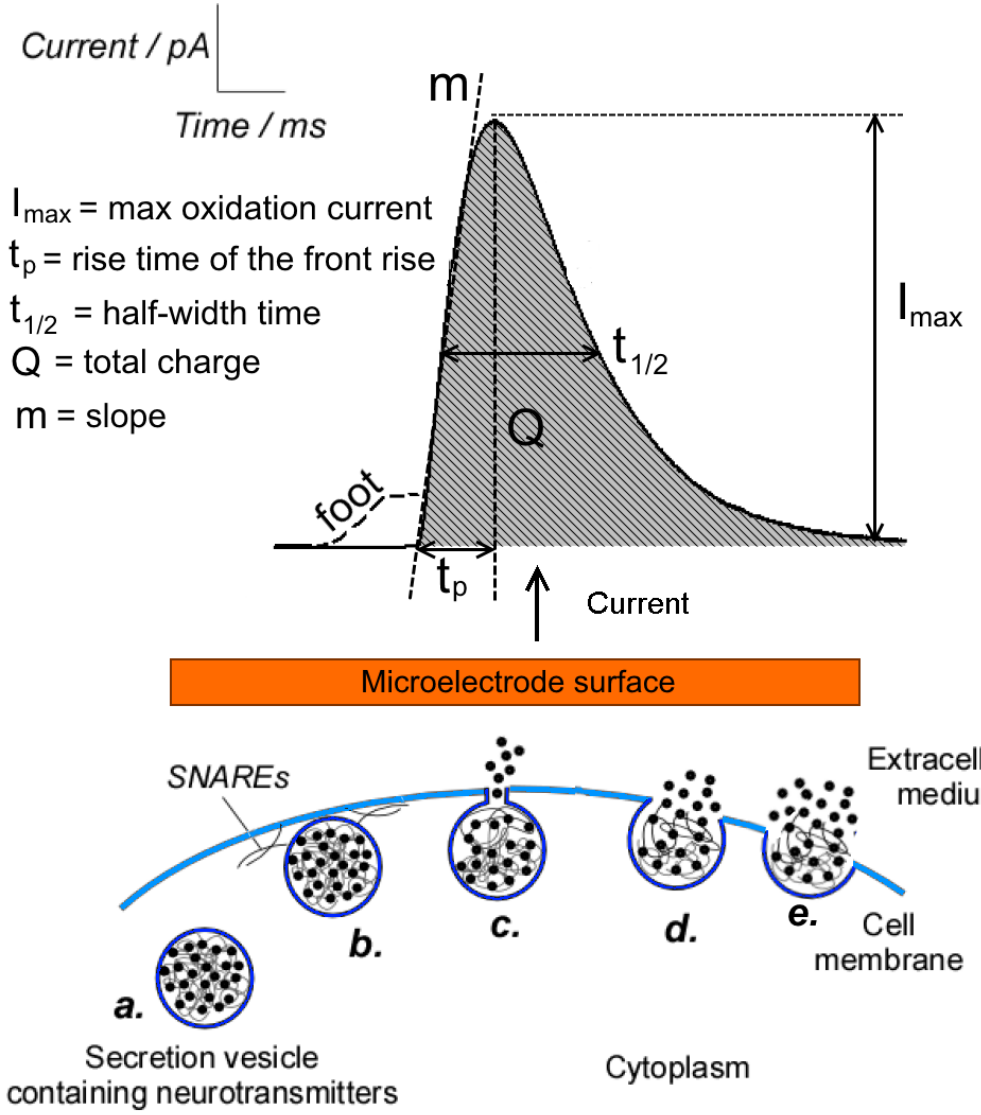
Improve Ca channels kinetics

Amperometric detection of exocytosis



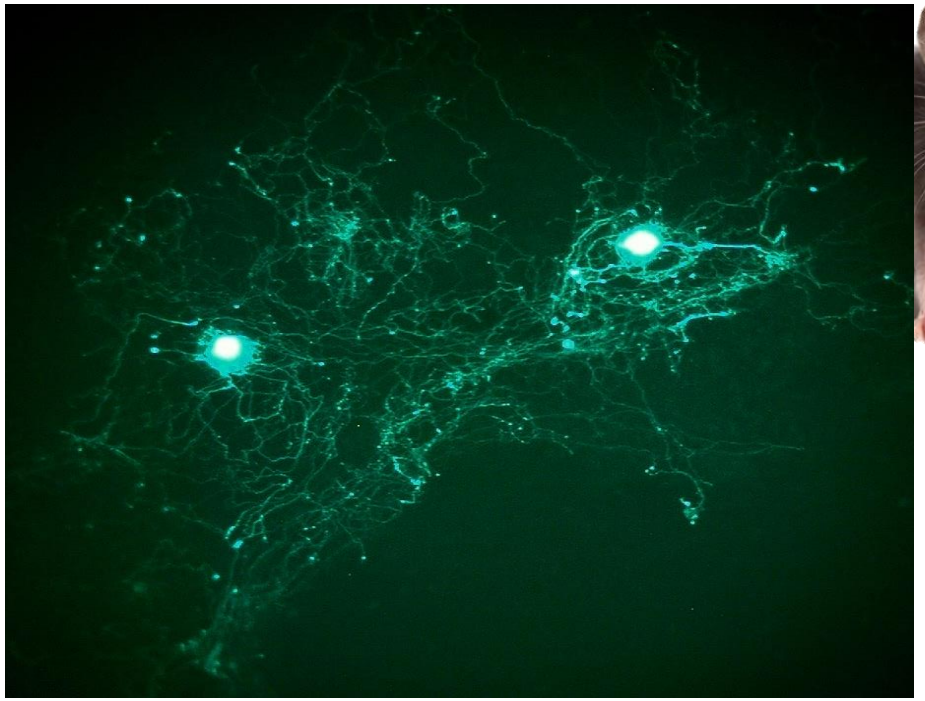
Adrenaline oxidation

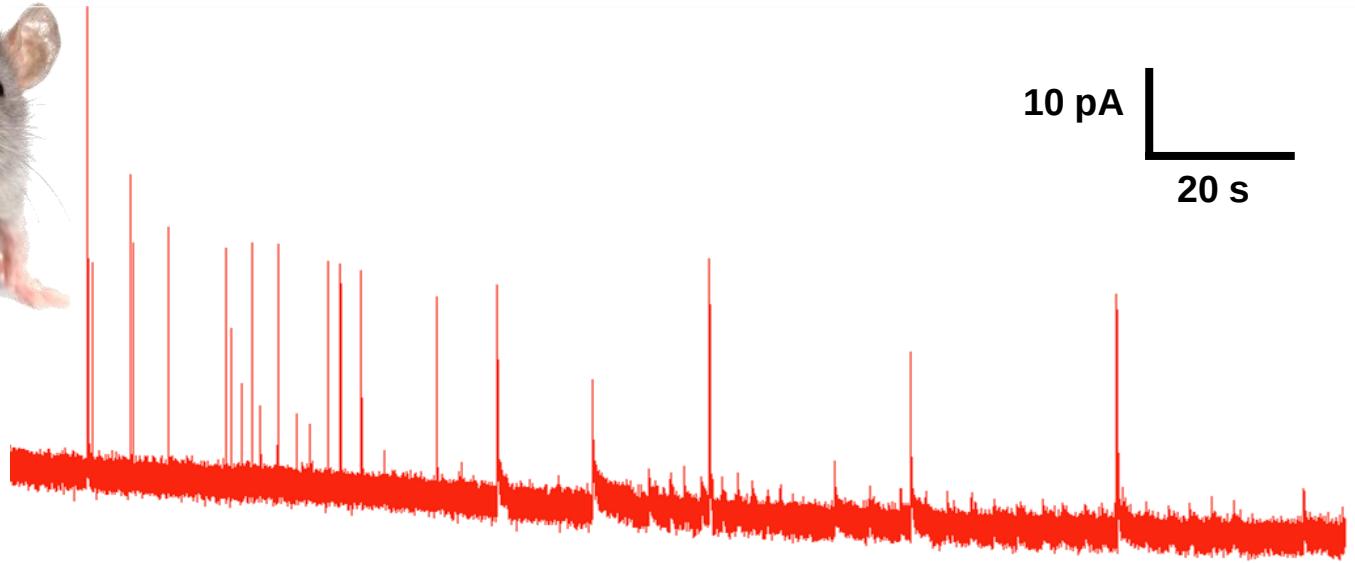
- **secretion of catecholamines** (adrenaline, noradrenaline, etc.)
- catecholamines are **secreted from vesicles** in which they are highly concentrated → **strong signal**
- secretion from 1 vesicle: 50-100 ms
- **detection of the oxidized species** in correspondence of a biased electrode
- **electrically or chemically stimulated**



F. Lemaître et al., **ElectrochimicaActa** 140,457(2014)

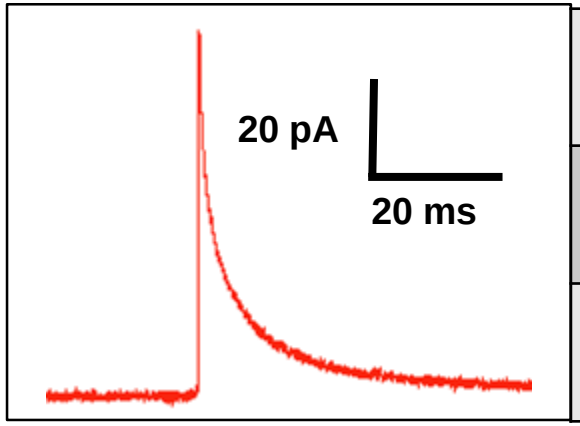
Exocytosis detection from *substantia nigra* neurons





Network of *substantia nigra* neurons

- Experiment performed after 21 DIV
- Cell network threated with L-Dopa for 1 h
  → increasing of vesicles dimension
- Stimulation with KCl solution

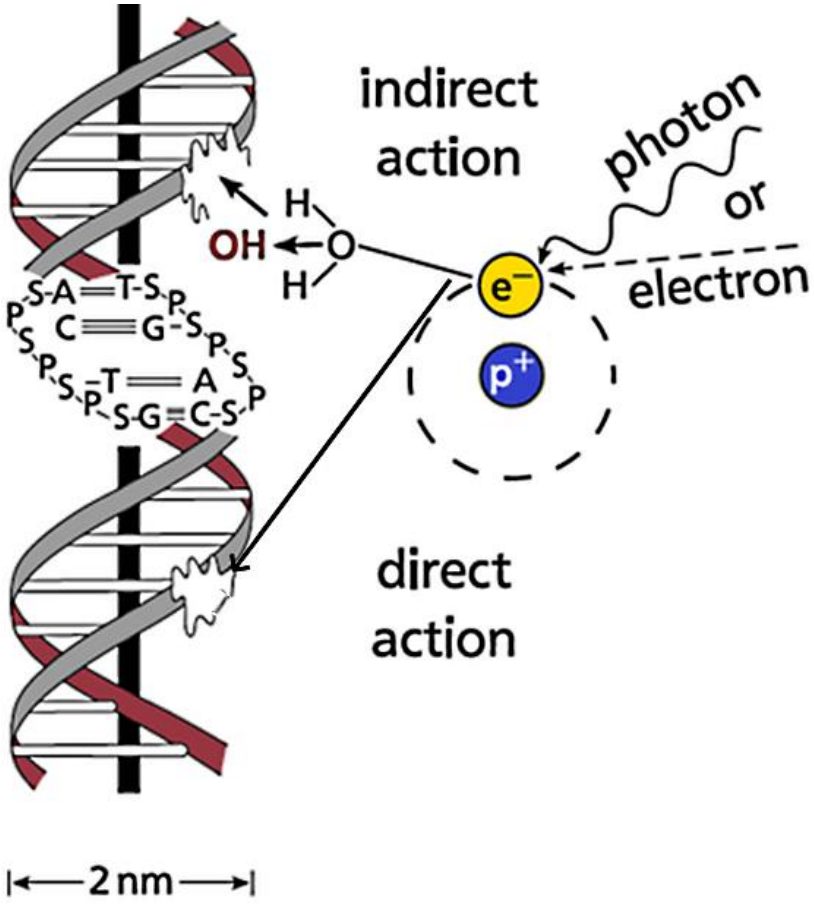


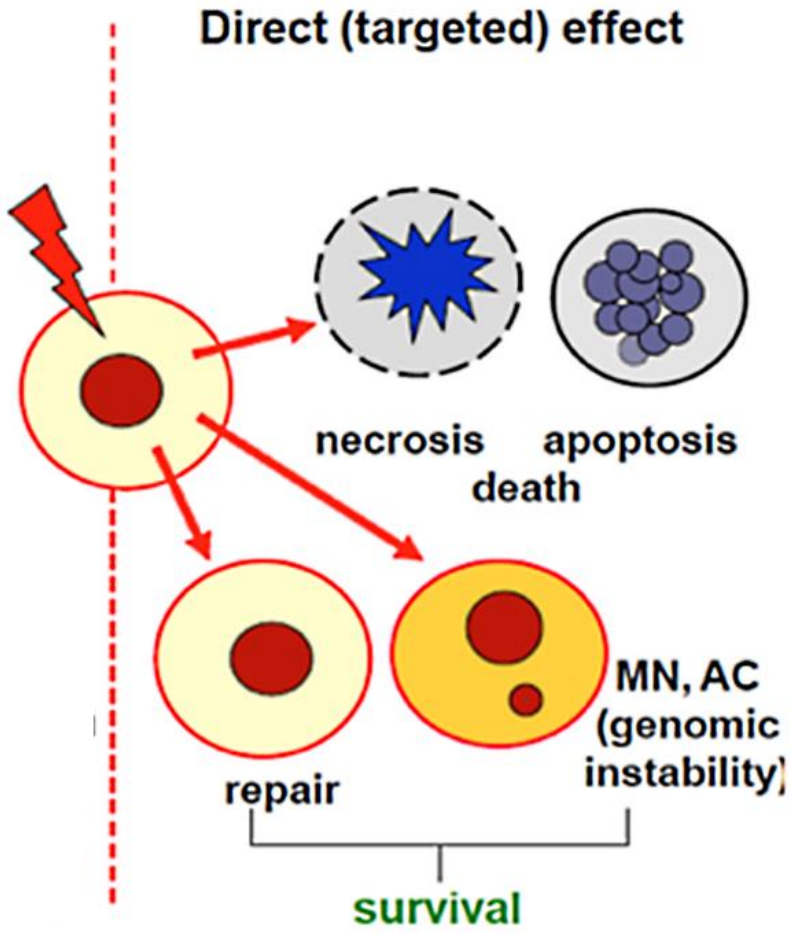
$t_{1/2}$ (ms)	2.89 ± 0.15
I_{max} (pA)	37.4 ± 1.5
Q (pC)	1.1 ± 0.4

DIAMOND BIOSENSORS for RADIOBIOLOGY

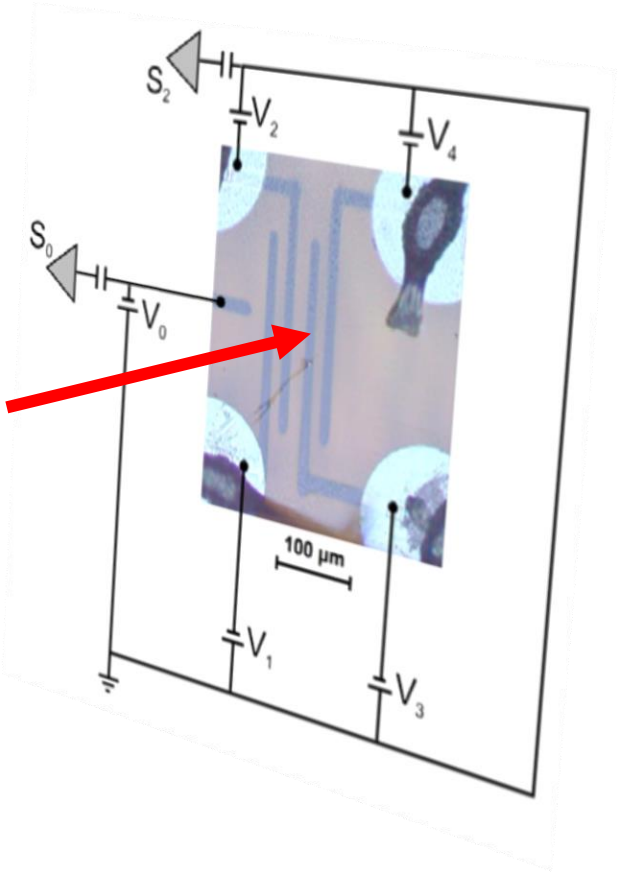
Radiobiology

Branch of biophysics concerned with the effects of ionizing radiation on organisms

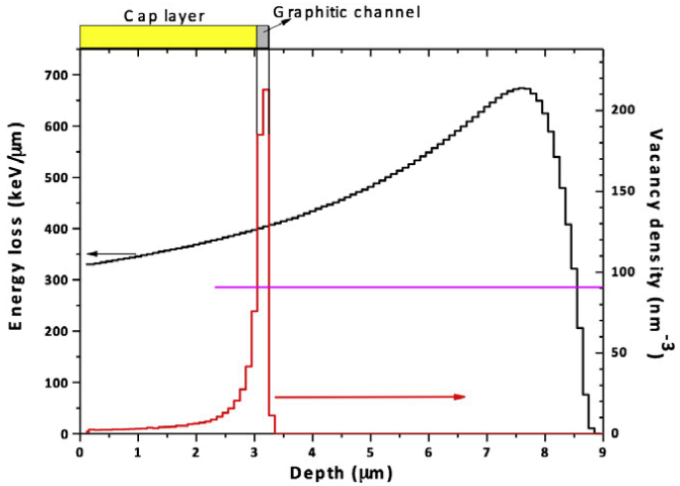


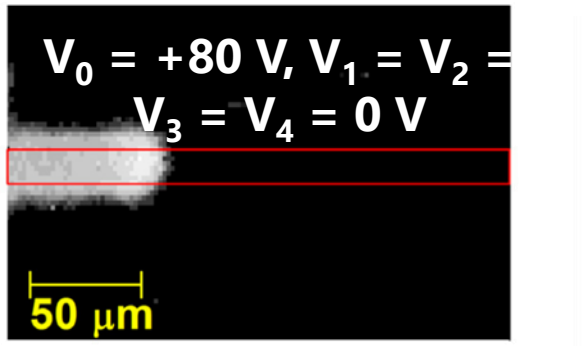


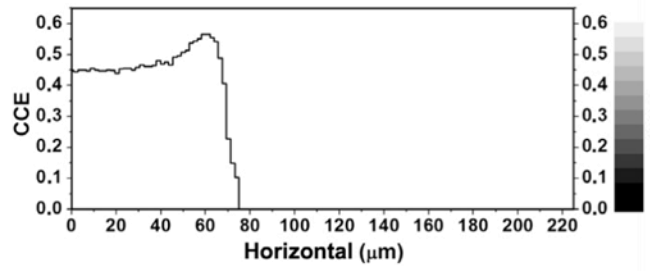
Ionizing radiation detection

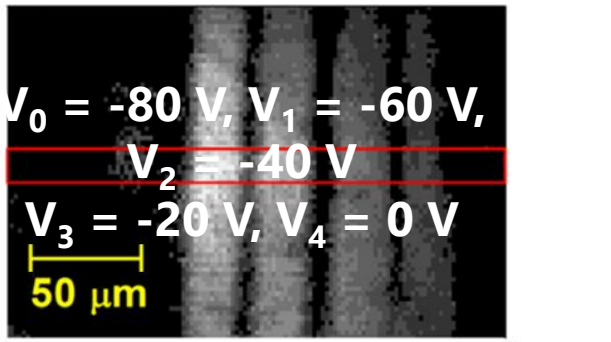


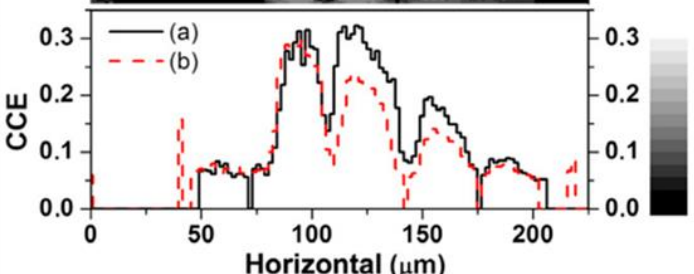
IBIC characterization of interdigitated particle detector





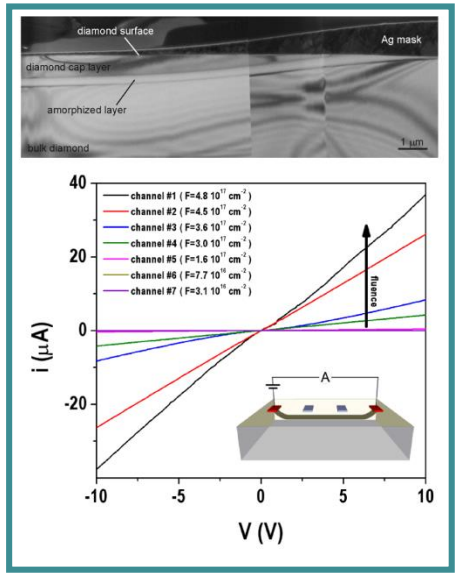




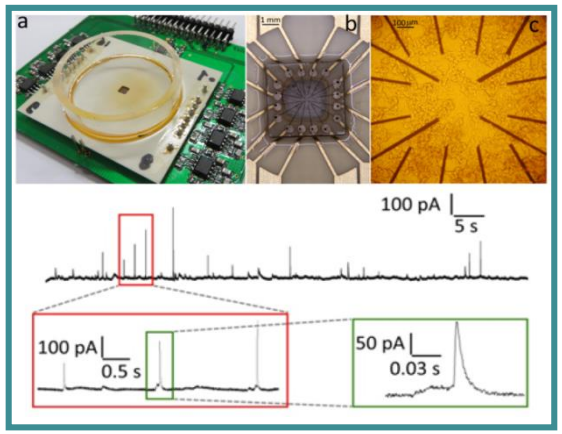


DIACELL project

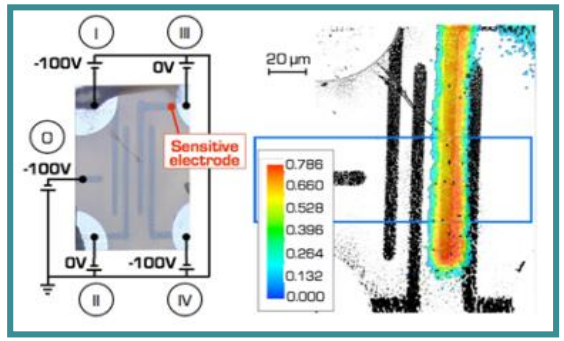
Diamond substrate: robust & reproducible, bio-compatible, non-toxic, optically transparent, tissue equivalent, radiation hard



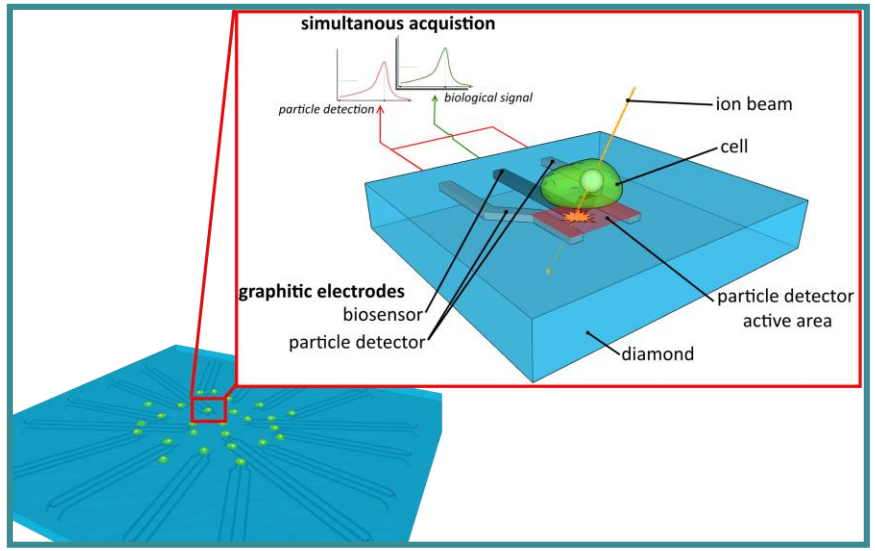
Deep ion beam lithography



Cellular bio-sensors

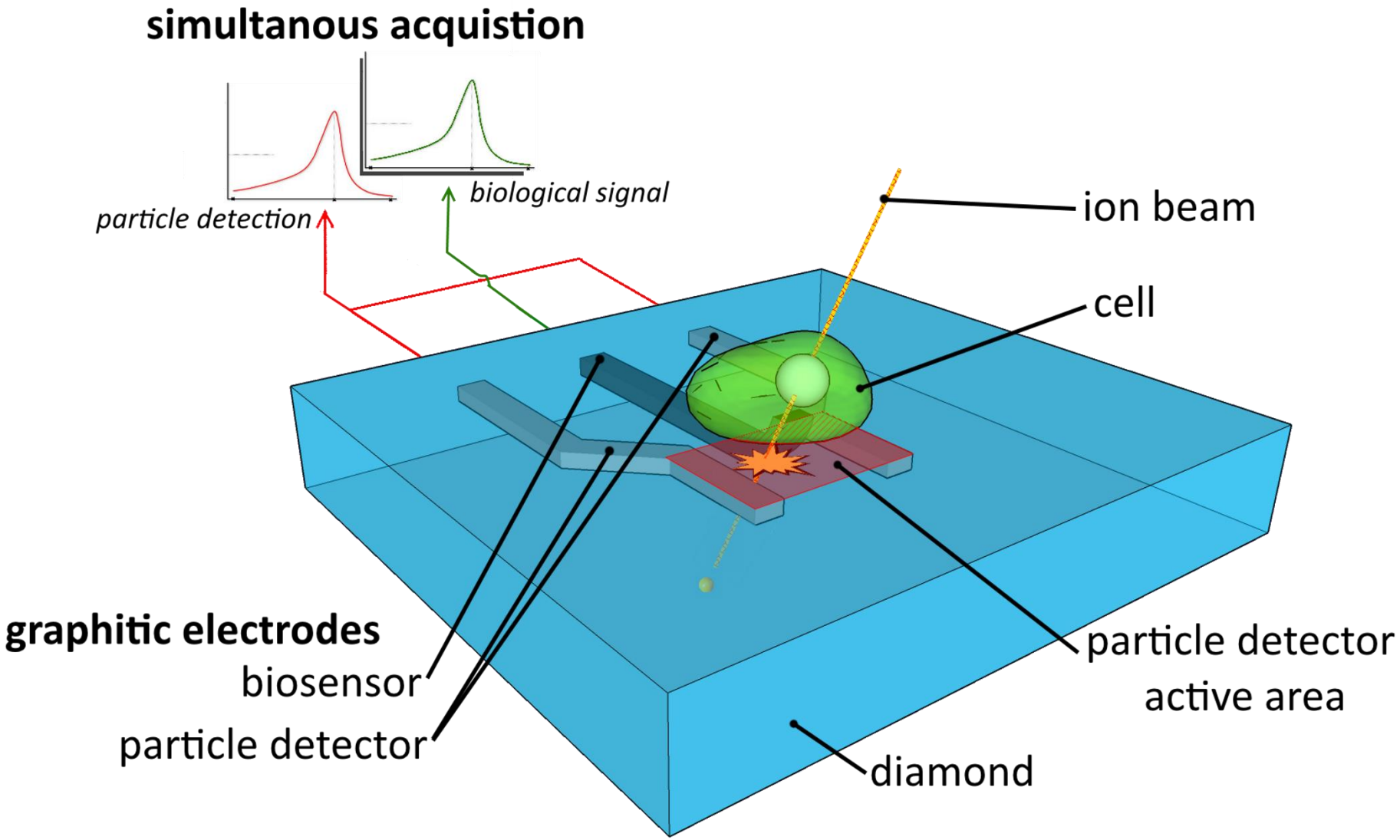


Radiation detectors

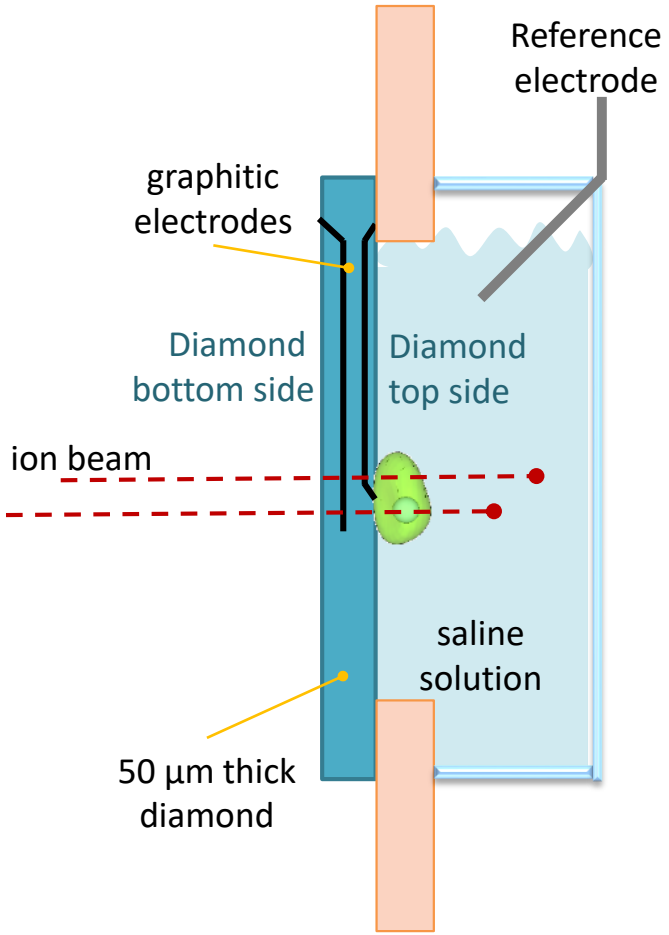


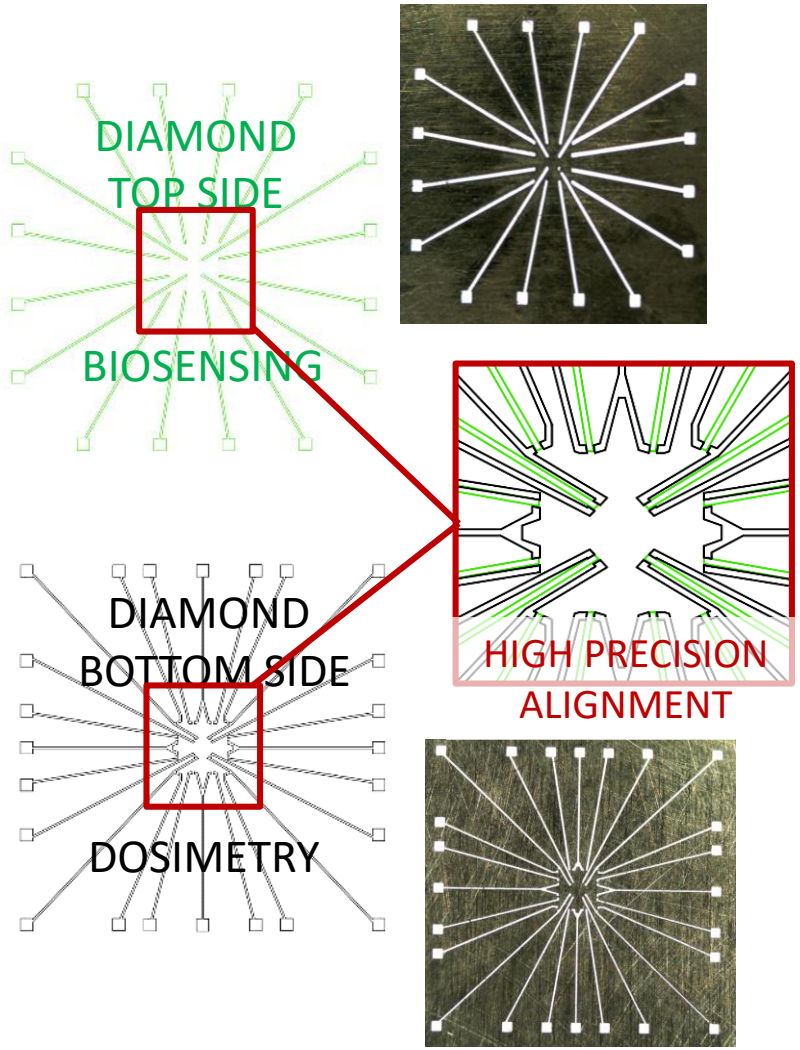
Integrated device

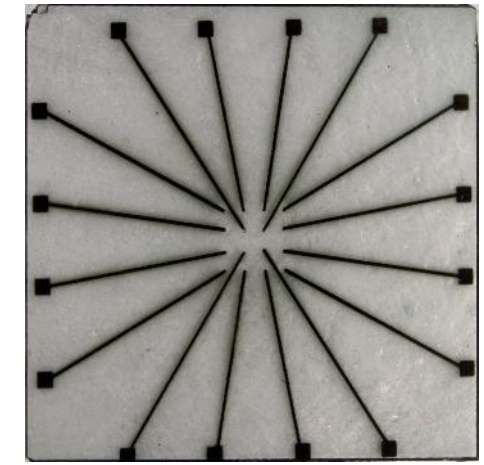
Diamond based-detector for radiobiology



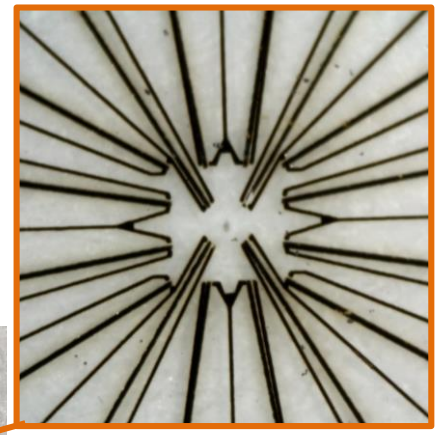
Sensor fabrication by means of IBL

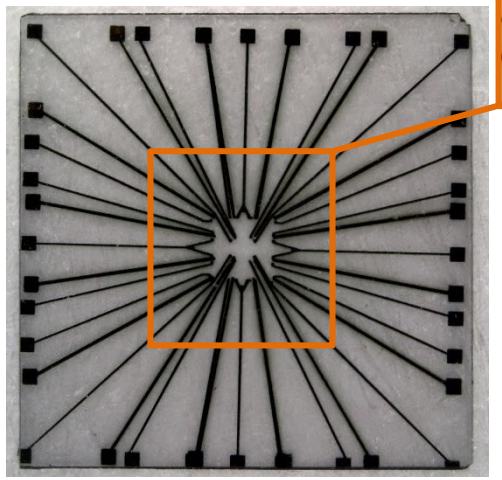






Optical micrographs

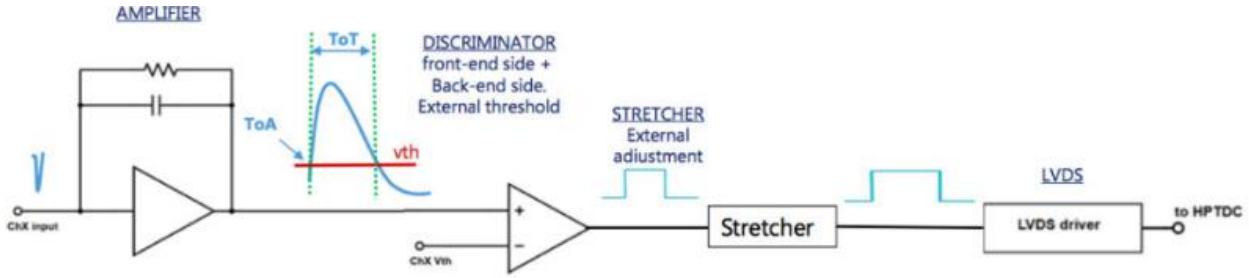




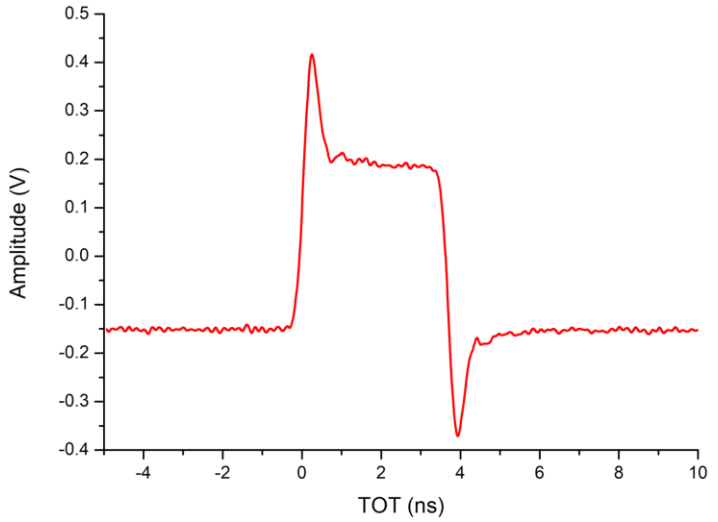
- ✓ Vertical irradiation
- ✓ Thin detector grade diamond

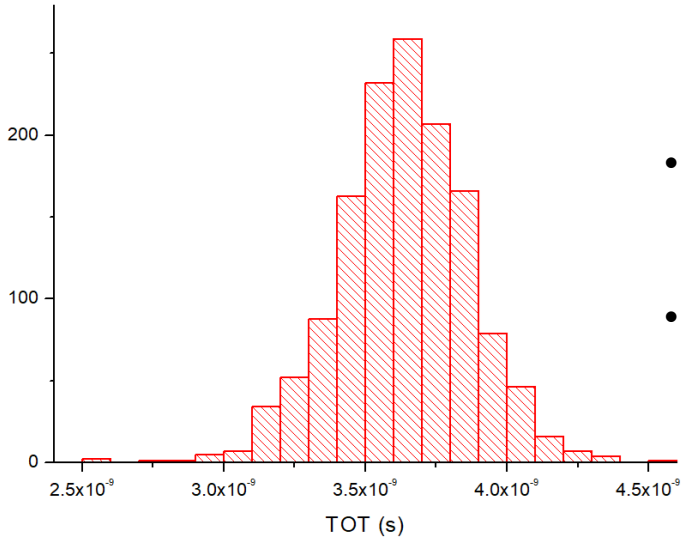
Sensor characterization: dosimetry

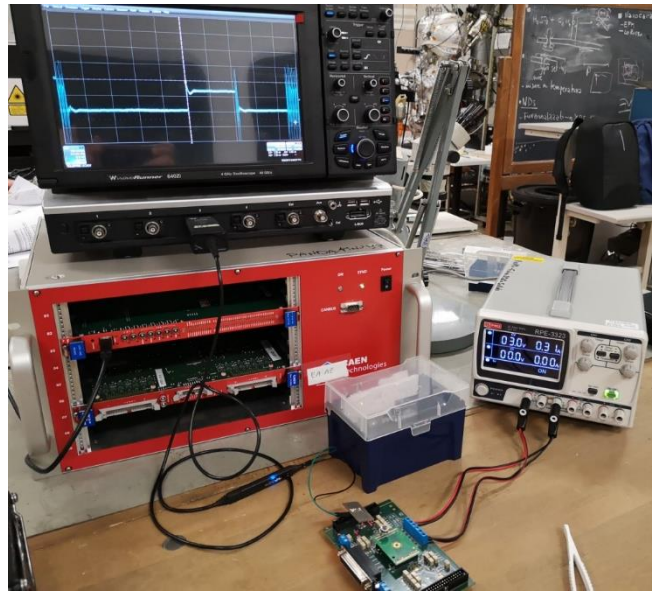
Interfacing of diamond sensors with TOFFEE (in collaboration with UFSD)



- ✓ Amplification stage
- ✓ Variable threshold discriminator
- ✓ LVDS output

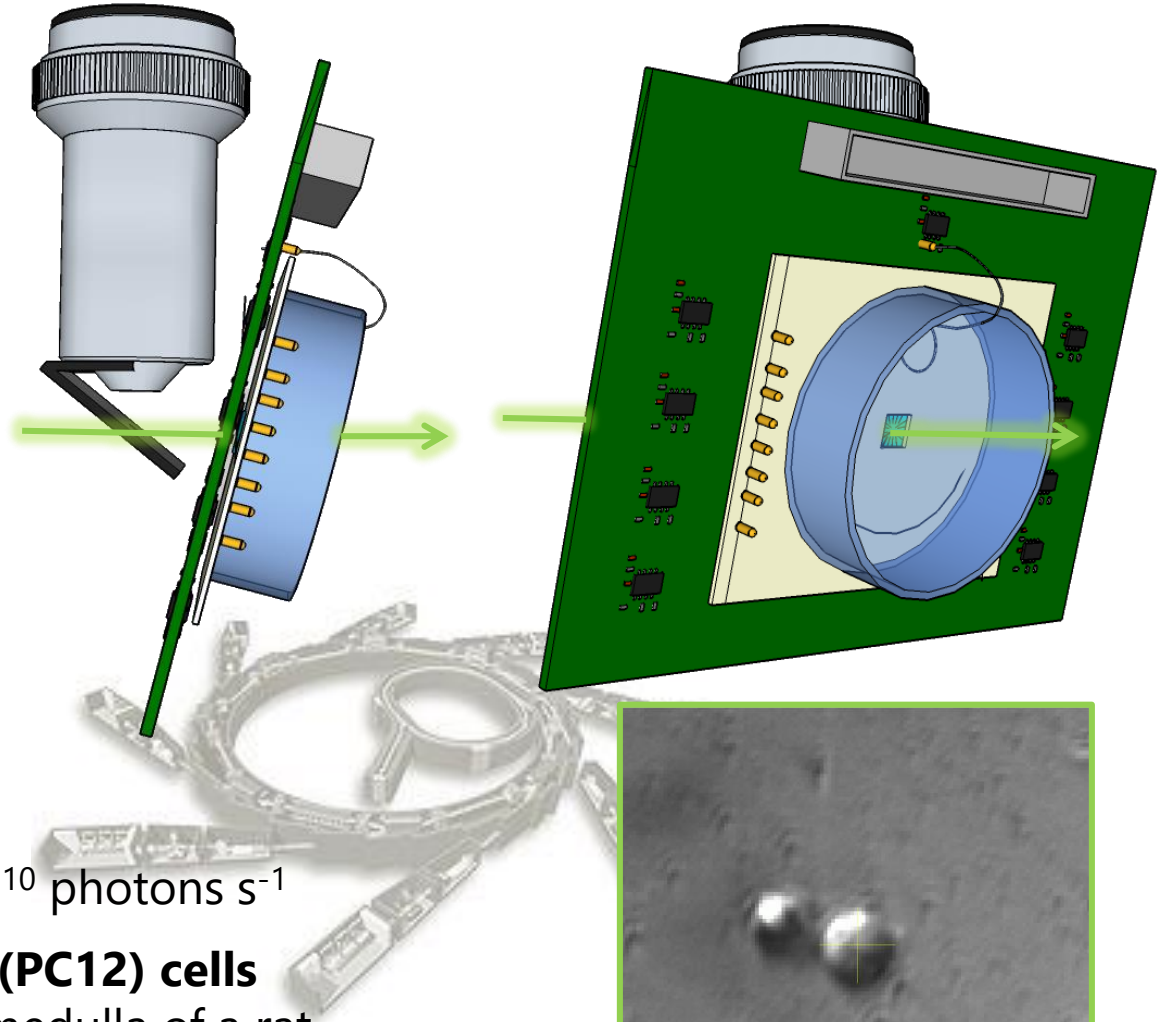


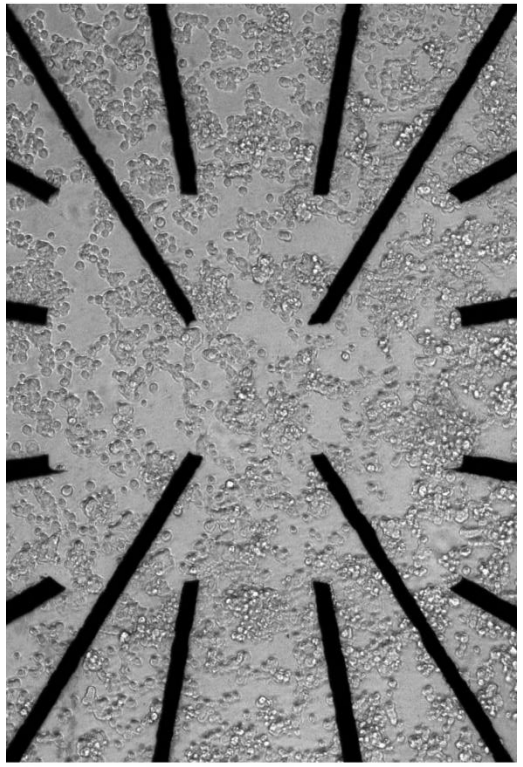


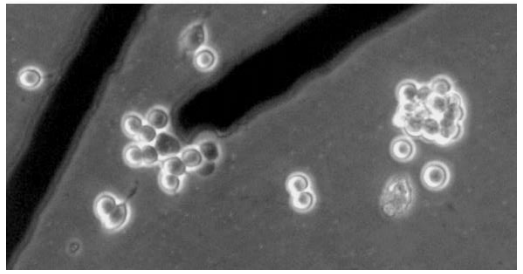


- Electronic chain already interfaced with graphitic electrodes in diamond
- Detection test performed both with X-Ray and alpha particles

Synchrotron X-Ray nano-beam cells irradiation







ID 16 nanobeam line

- 200 nm spot size
- E = 17.4 keV; $\Phi = (0.1 – 1)\cdot 10^{10}$ photons s^{-1}

Adrenal phaeochromocytoma (PC12) cells

- cancer cell line from adrenal medulla of a rat
- model for neurosecretion studied

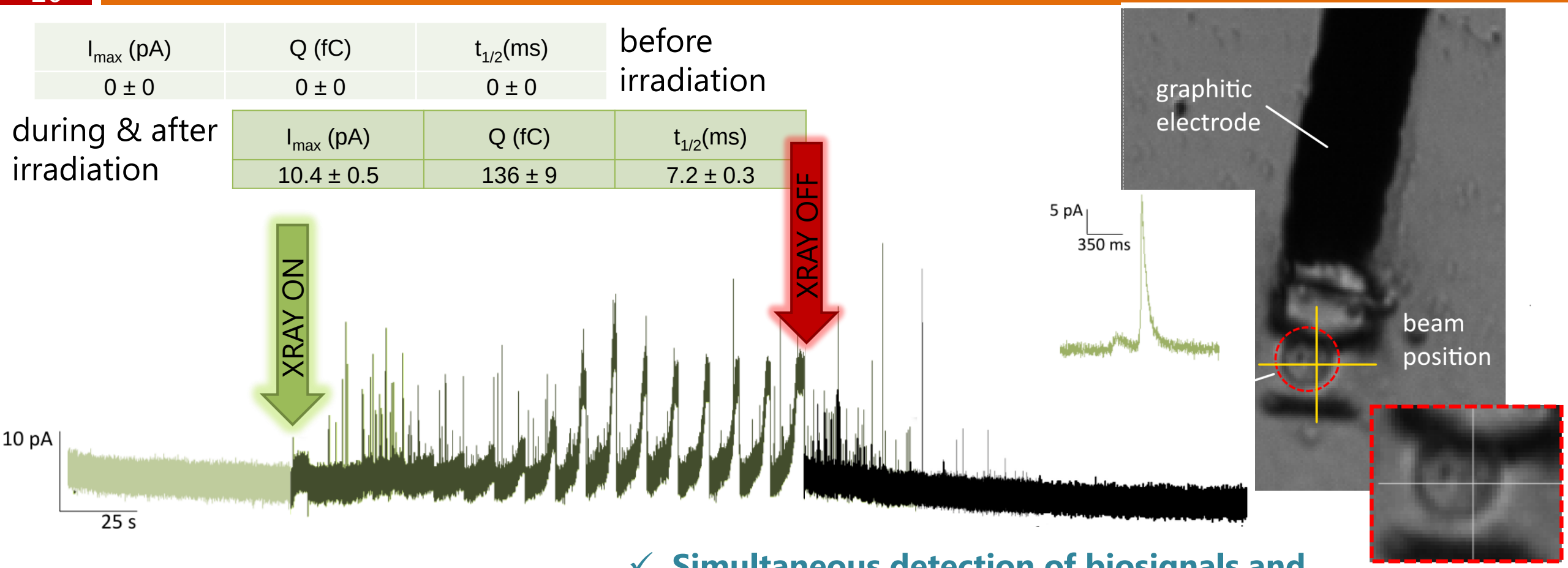
X-Ray induced exocytosis

before irradiation

I_{max} (pA)	Q (fC)	$t_{1/2}$(ms)
0 ± 0	0 ± 0	0 ± 0

during & after irradiation

I_{max} (pA)	Q (fC)	$t_{1/2}$(ms)
10.4 ± 0.5	136 ± 9	7.2 ± 0.3



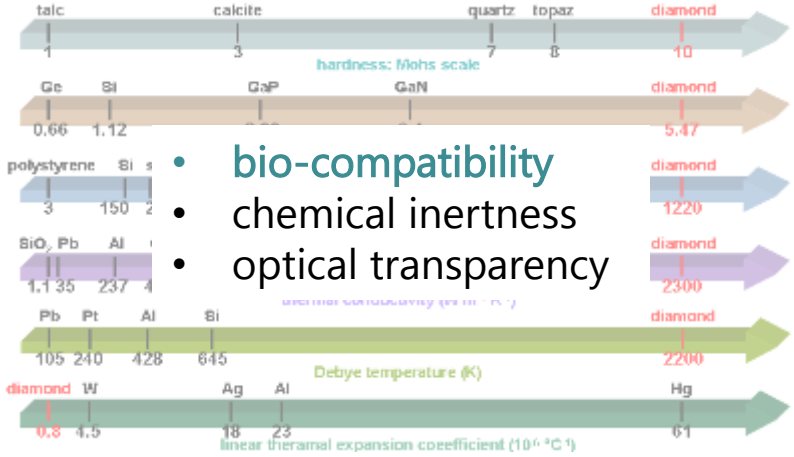
control

I_{max} (pA)	Q (fC)	$t_{1/2}$(ms)
8.75 ± 0.18	175 ± 4	3.67 ± 0.17

- ✓ **Simultaneous detection of biosignals and ionizing radiation**
- ✓ **First observation of single cell exocytosis stimulation with X-Ray**

NANO-DIAMOND for BIOSCIENCE

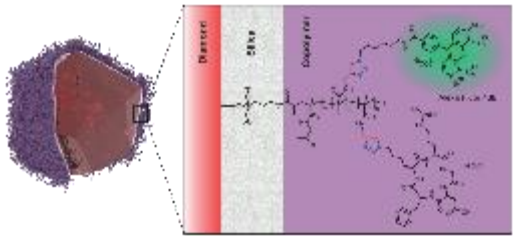
Nanodiamond properties *#2*



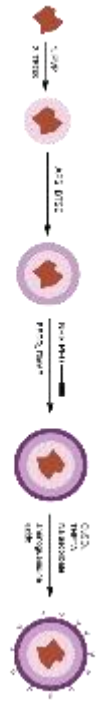
- **bio-compatibility**
- chemical inertness
- optical transparency

Surface modification

Chemical functionalization



J. Slegerova, et al., **Nanoscale**, 7 , 415 (2015)



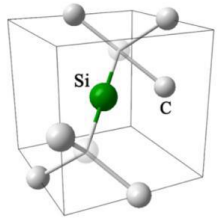
I. Rehor, et al., **Small**, 10, 6, 1106 (2014)

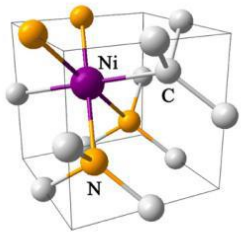
Thermal processes

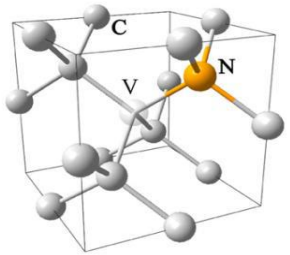
- graphitization
- oxidation
- hydrogenation
- …

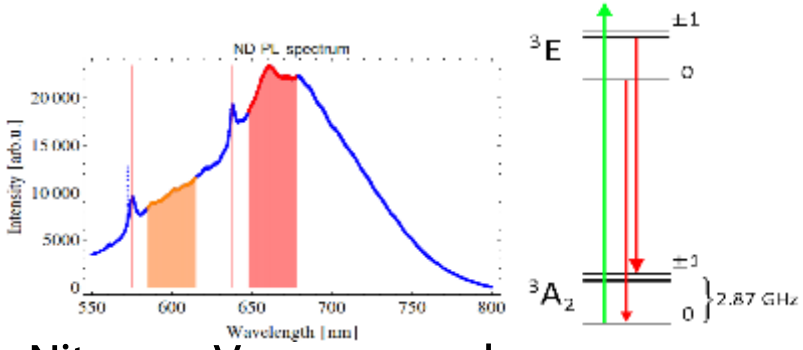
Luminescent lattice defects:

- Vacancies
- Substitutional
- Interstitials



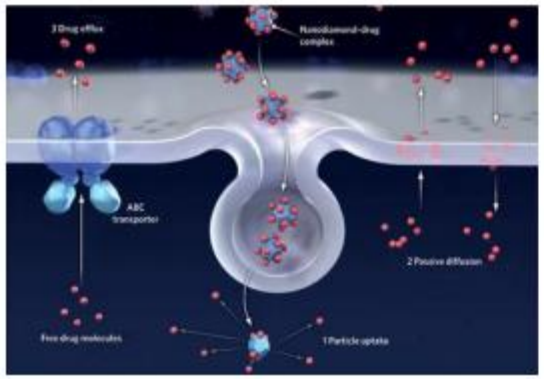


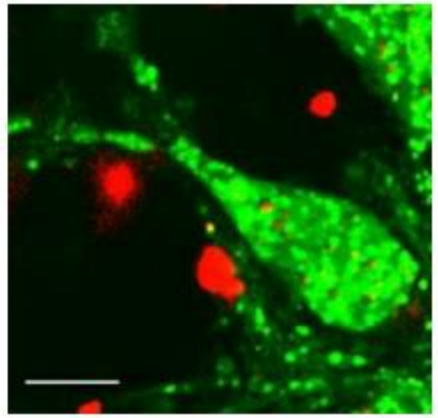




Nitrogen-Vacancy complex

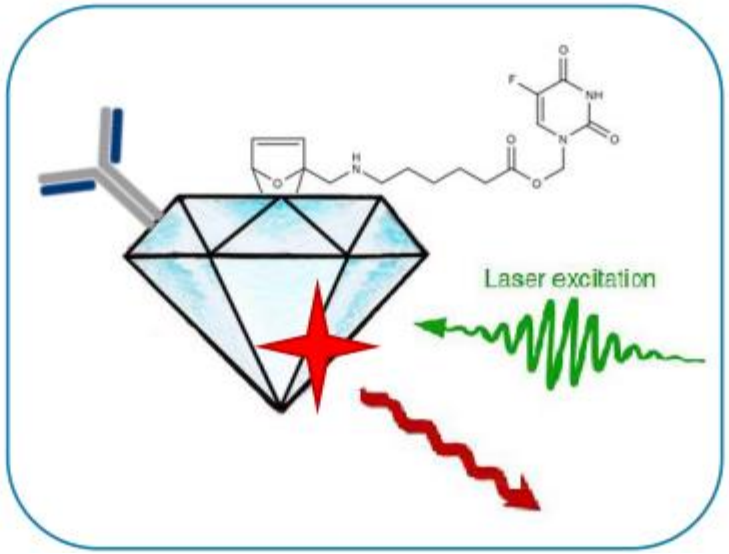
Multifunctional nano-particles





Drug Delivery:

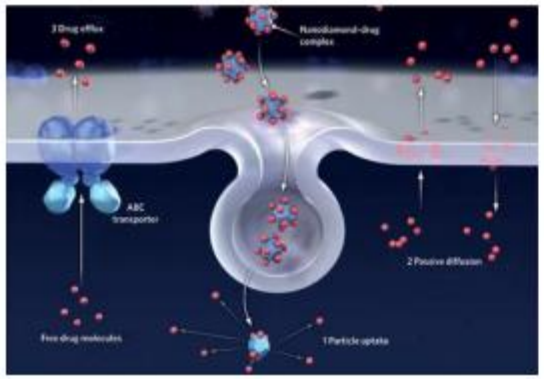
veicolazione farmaci in target cellulari specifici



Luminescenza:

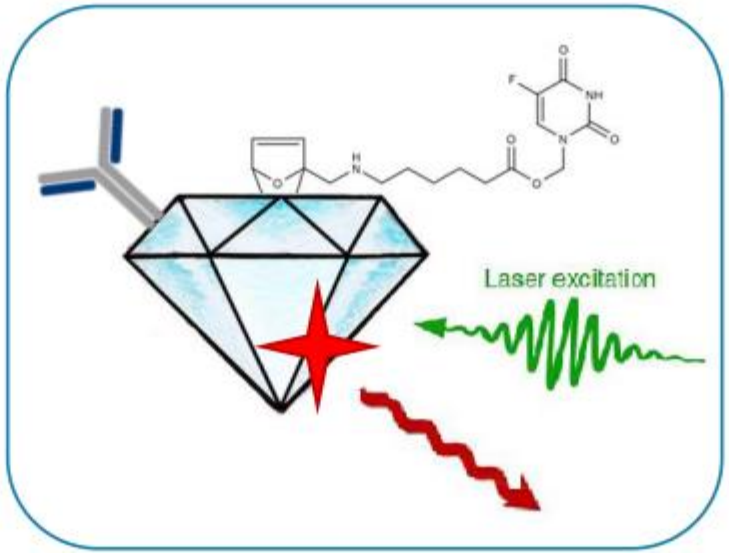
possibilità di tracciare le nanoparticelle nel processo biologico

Multifunctional nano-particles



Drug Delivery:

veicolazione farmaci in target cellulari specifici



Luminescenza:

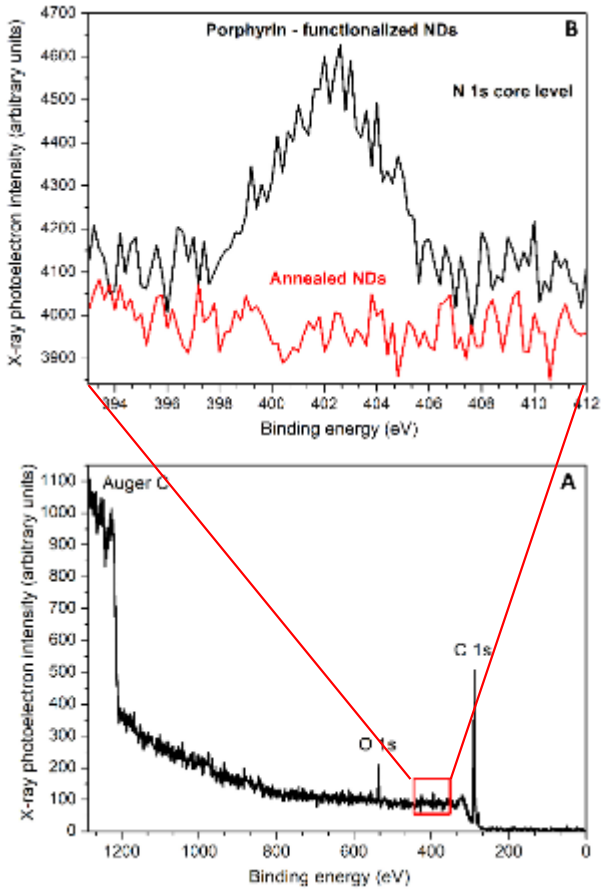
possibilità di tracciare le nanoparticelle nel processo biologico

Nanodiamonds surface modification

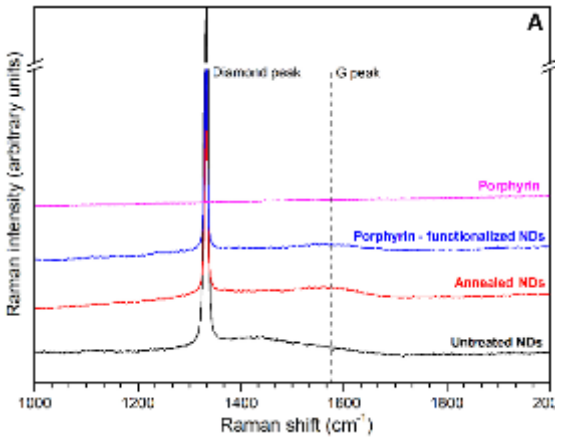
Sample preparation:

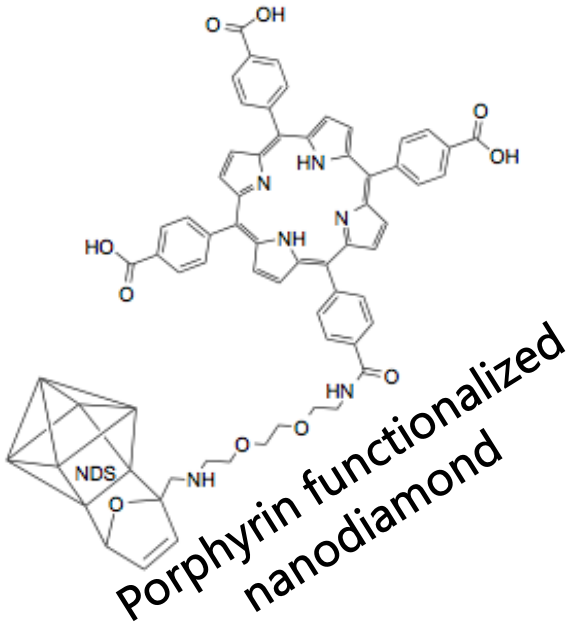
- thermal annealing
  800 °C × 8 h in vacuum

XPS

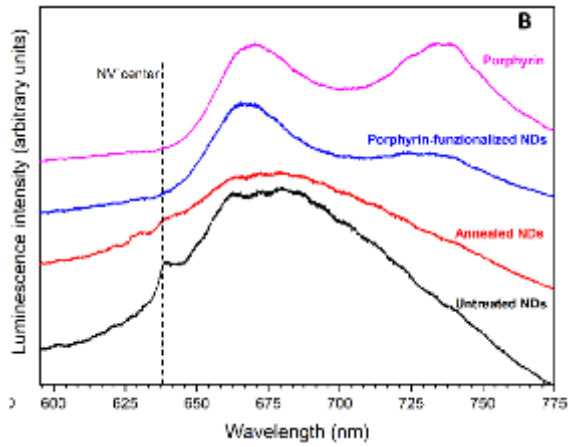


Raman

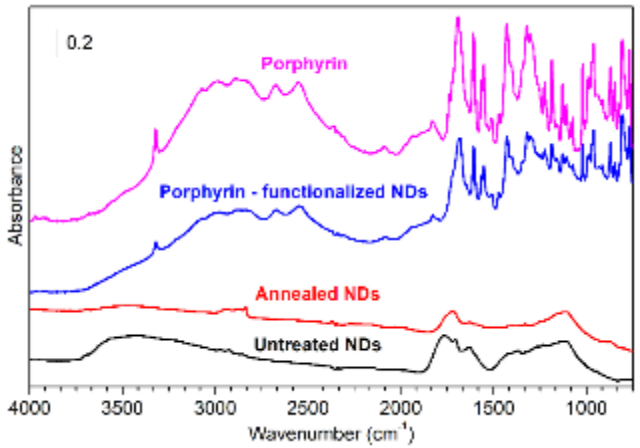




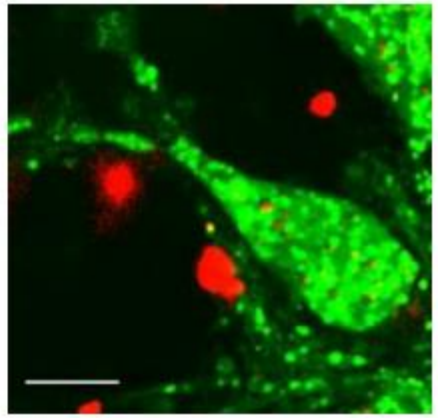
PL



DRIFT

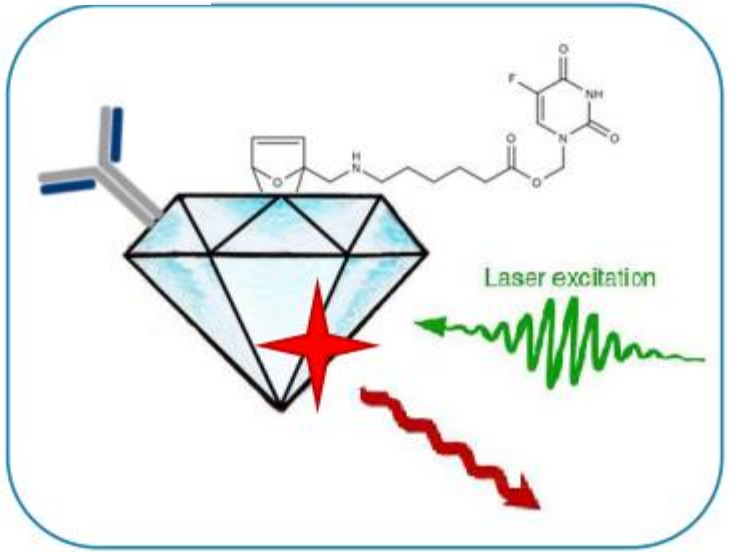


Multifunctional nano-particles



Drug Delivery:

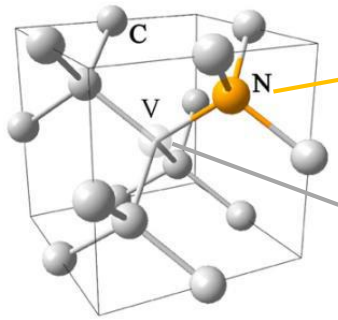
veicolazione farmaci in target cellulari specifici



Luminescenza:

possibilità di tracciare le nanoparticelle nel processo biologico

MeV ion induced damage in nanodiamond



Nitrogen already present in the crystal (100 ppm).

Vacancies created by MeV ion implantation

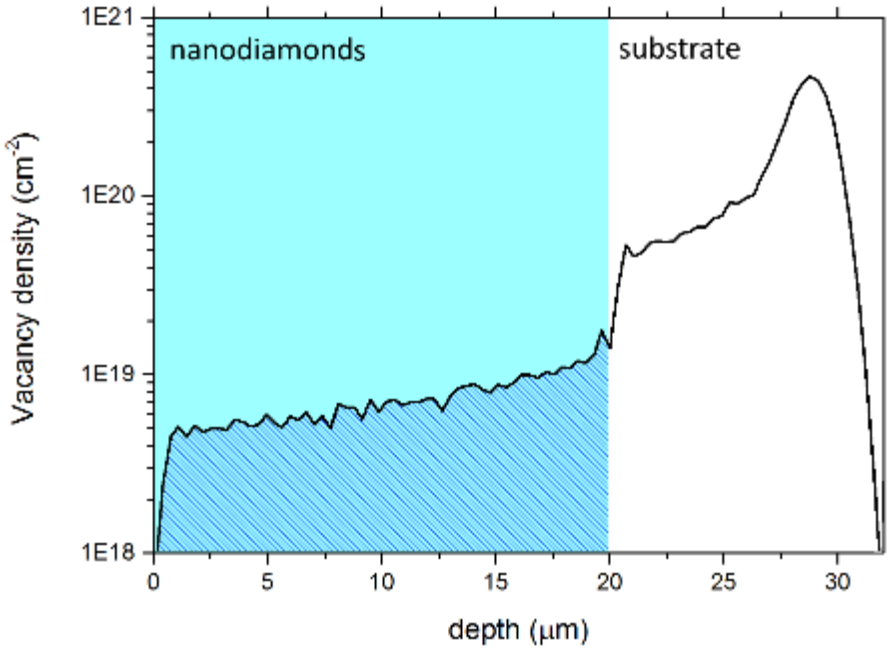
implantation:

- H^+ @ 2 MeV
- penetration depth ~25 μm
- fluence $5 \cdot 10^{15}$ cm^{-2}

$$\rho_V = \lambda_V \cdot F = 5 \cdot 10^{18} \#_{vac}\ cm^{-2}$$

$\lambda = 10^3\ \#_{vac}\ \#_{ion}^{-1}\ cm^{-1}$

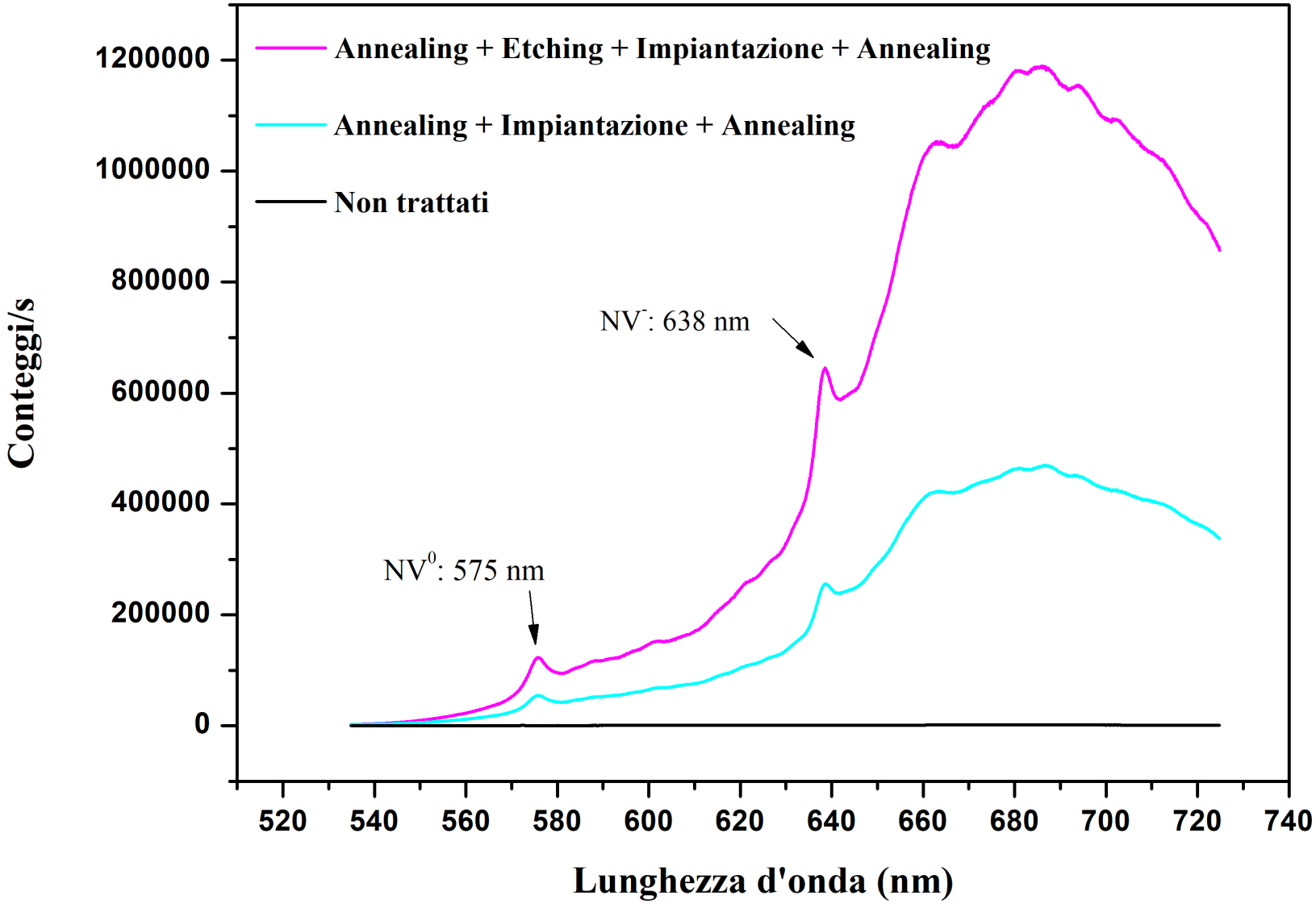
~ tens of NV center also in smaller crystal



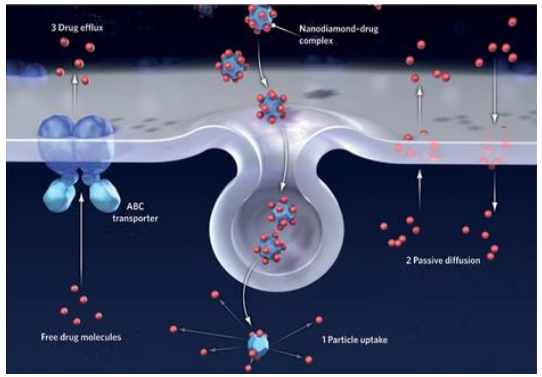
thermal treatment:

- 800 °C for 1 hours
- 800 mbar in N_2

Photoluminescence spectra

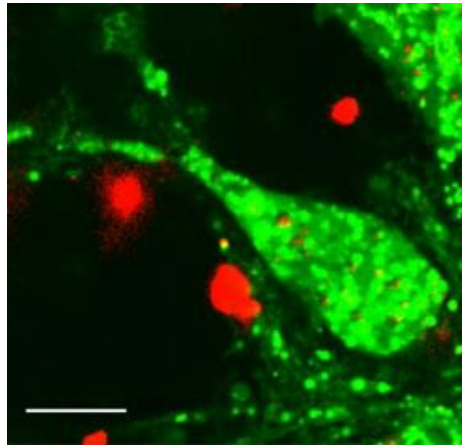


	Confronto	Valore
1	$\frac{I_{685nm}(ann + etch + imp + ann)}{I_{685nm}(non\ trattati)}$	770 ± 20
2	$\frac{I_{685nm}(ann + imp + ann)}{I_{685nm}(non\ trattati)}$	310 ± 20
3	$\frac{I_{685nm}(ann + etch + imp + ann)}{I_{685nm}(ann + imp + ann)}$	2.506 ± 0.004

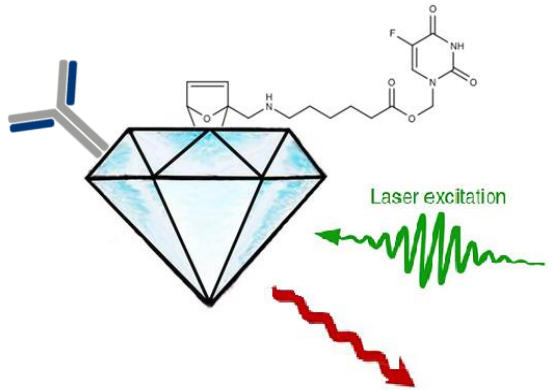


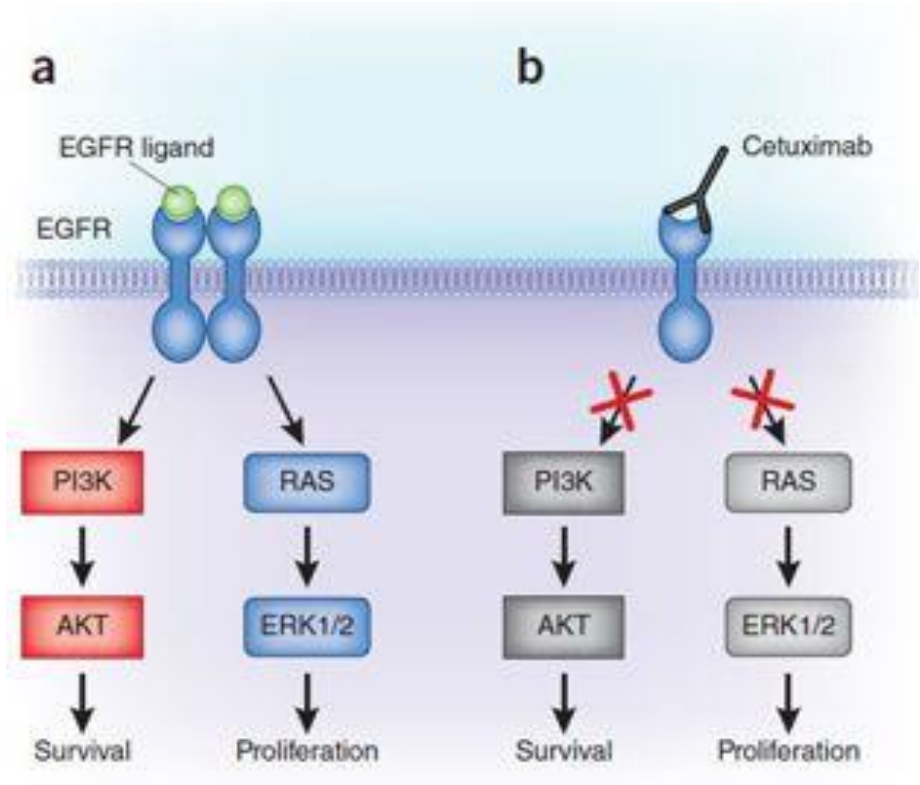
Drug Delivery

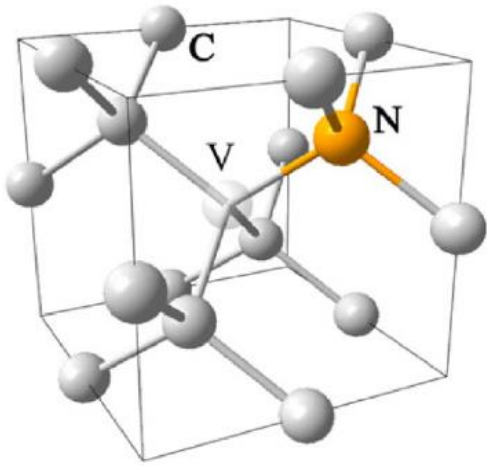
(CETUXIMAB)



Luminescenza



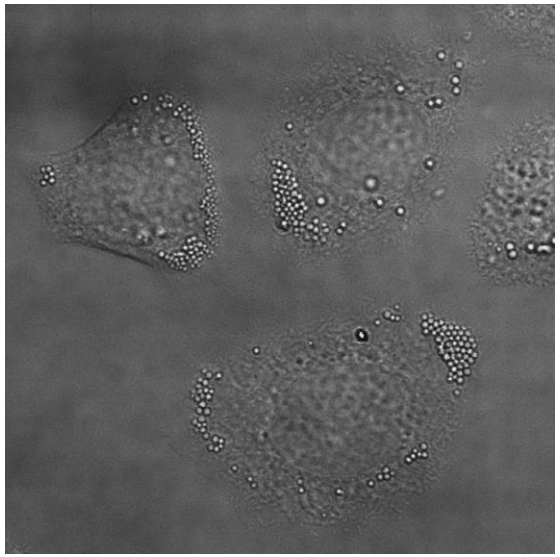


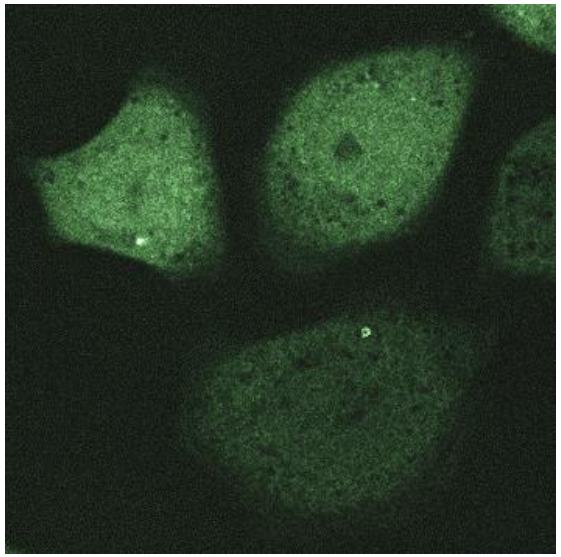


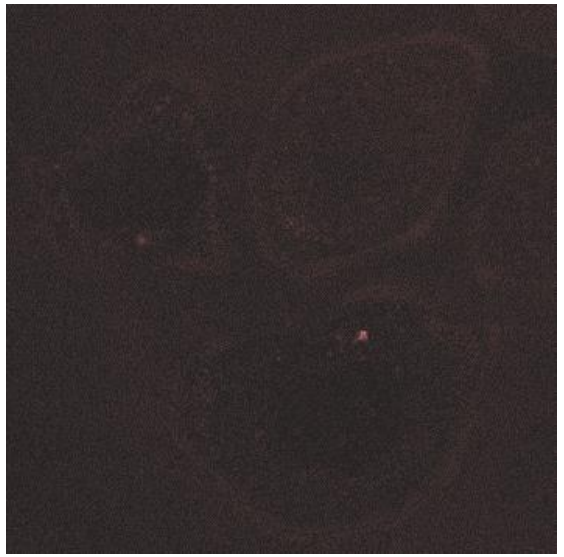
Confocal microoscopy

CONTROL

Only Nanodiamond

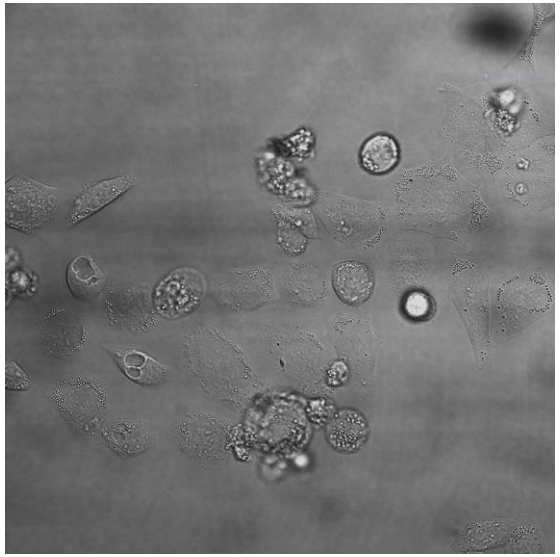


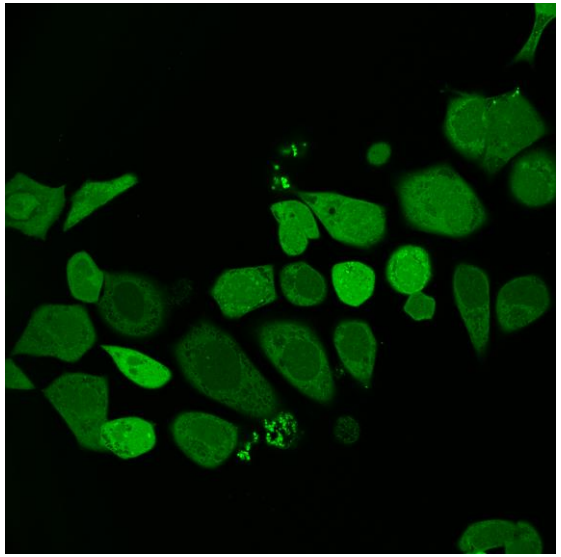


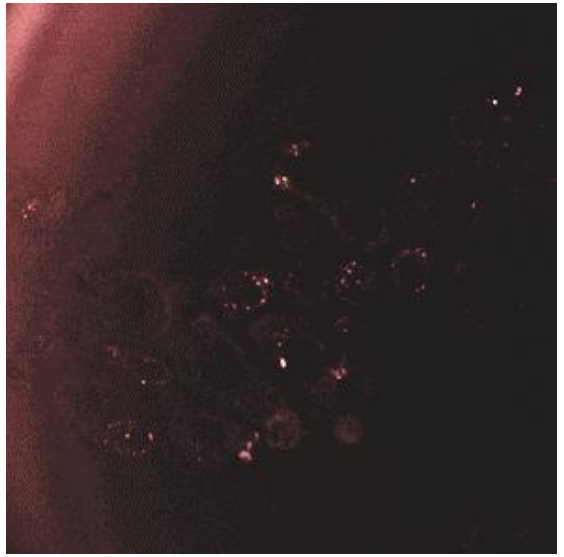


Modified Nanodiamond

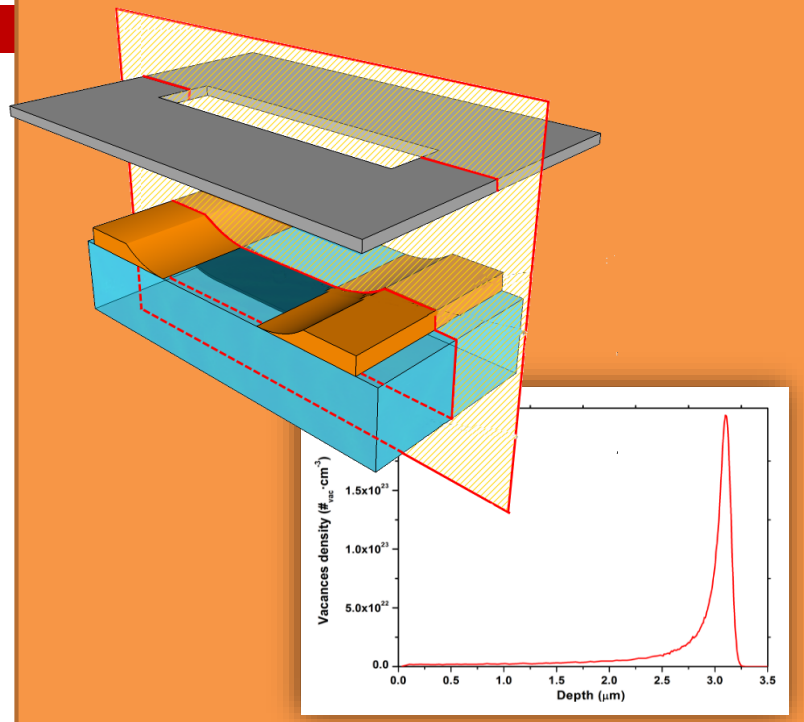
ND + CETUXIMAB



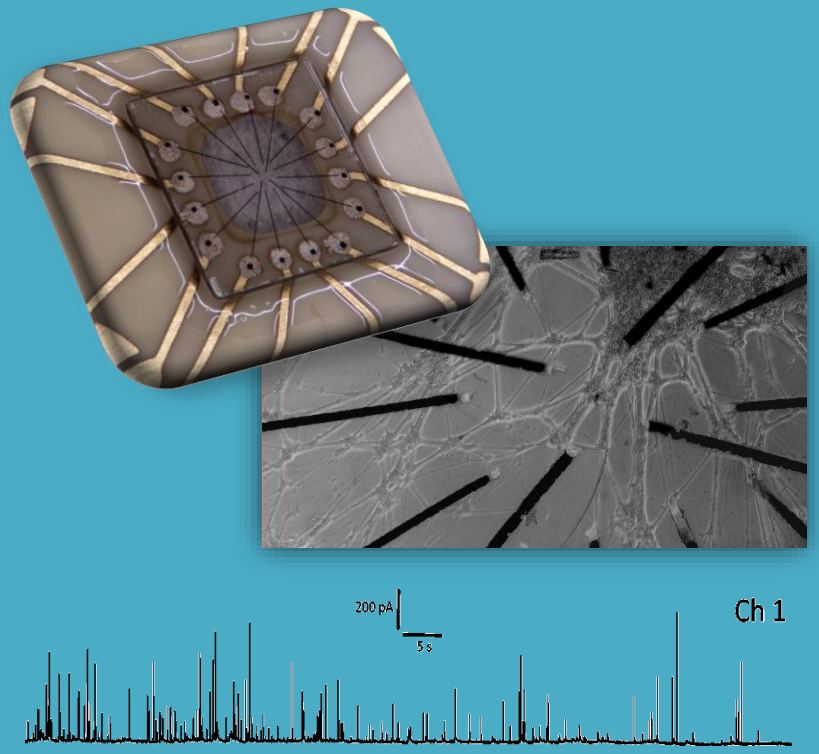




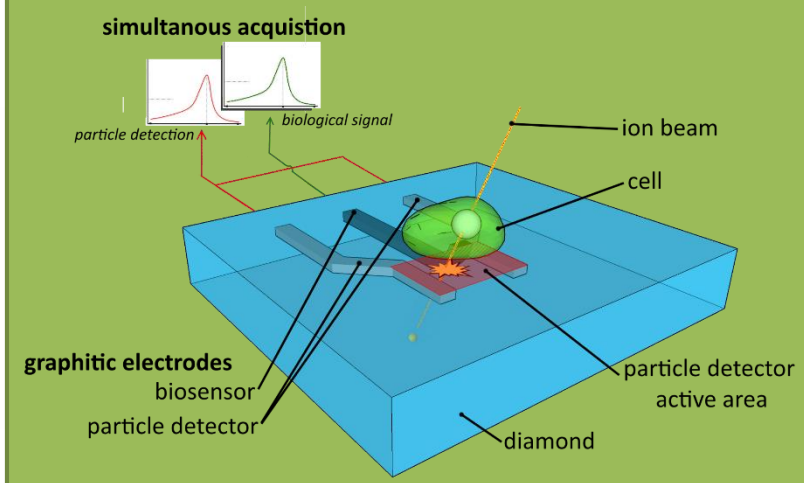
MeV ion beam lithography of diamond



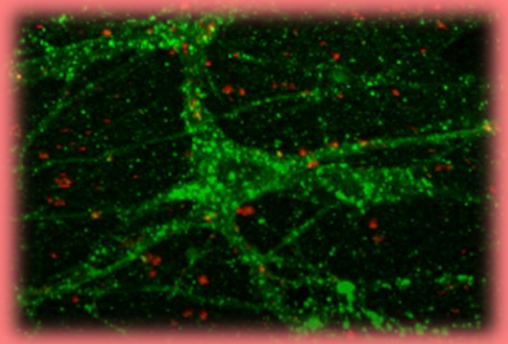
Multi electrodes cellular bionsensor



Simultaneous detection of ionizing radiation and biosignals



Multifunctional nanodiamond for drug-delivery



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