NATIONAL CANCER INSTITUTE

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Biomedical Physics Laboratory

Lithuanian National Cancer Institute Biomedical Physics Laboratory & Open Access Centre

Members of Biomedical Physics Laboratory

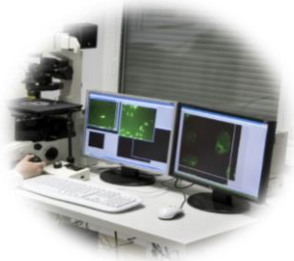
Head of the lab Prof. habil. Dr. **Ričardas Rotomskis**
Head of Open Access center Prof. Dr. **Vitalijus Karabanovas**
Senior Research Fellow Dr. **Simona Steponkienė**
Senior Research Fellow Dr. **Jonas Venius** (Open Access Center)
Senior Research Fellow Dr. **Greta Butkienė (Jarockytė)**
Research Fellow dr. **Juras Kišonas** (Open Access Center)
Junior Research Fellow PhD student **Evelina Kazlauskė (Voronovič)**
Junior Research Fellow PhD student **Marijus Plečkaitis**
Junior Research Fellow PhD student **Augustas Morkvėnas** (Open Access Center)
Junior Research Fellow PhD student **Džiugas Jurgutis**
Senior Specialist **Danutė Bulotienė**
Junior Research Fellow **Vilius Poderys**
Junior Research Fellow **Marius Stašys**

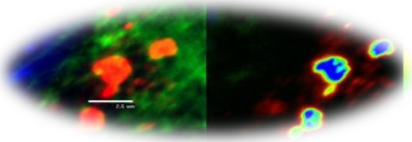
Main activities of the group

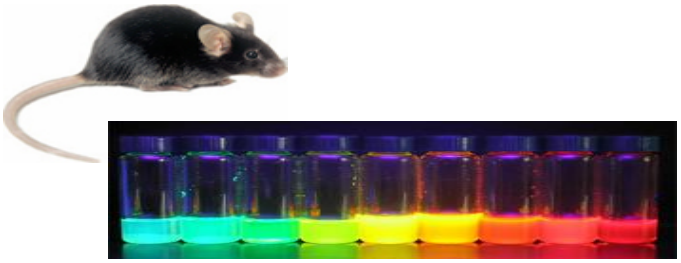
Research area *– development and improvement of novel methods and technologies for early diagnostics and combined treatment of cancer.*

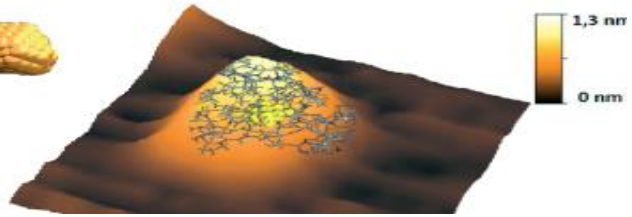
Directions:

- **Synthesis and modification of nanoparticles (NPs):** gold NPs and nanoclusters, quantum dots, magnetic NPs.
- **Physical characterisation of NPs**: optical spectroscopy, dynamic light scattering, atomic force microscopy, etc.
- ***In vitro*** **research** on NPs interaction with human cell lines: nanotoxicology, cellular distribution, NPs uptake pathways, biomolecule targetting etc.
- ***In vivo*** **research** on NPs effects in the organism: NPs stability and nanotoxicity, biodistribution, penetration through biobarriers, multimodal animal imaging (optical/CT/MRI), etc.
- **Clinical and preclinical research**: optical biopsy, *in vivo* reflectance confocal microscopy, smart illumination solutions.





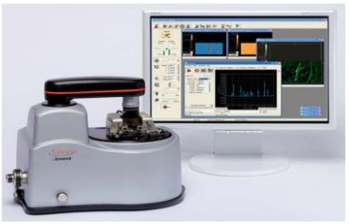
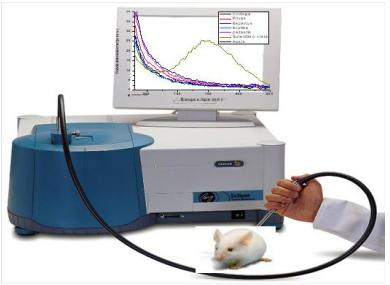


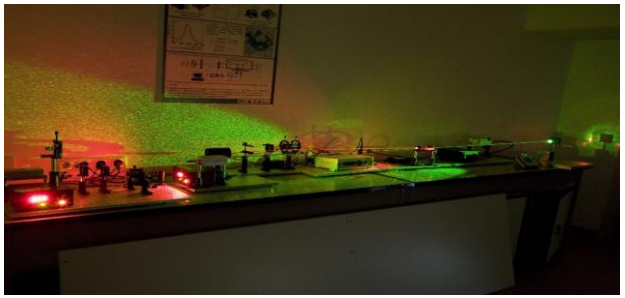
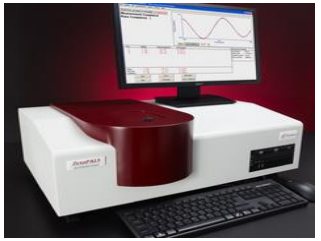


Infrastructure

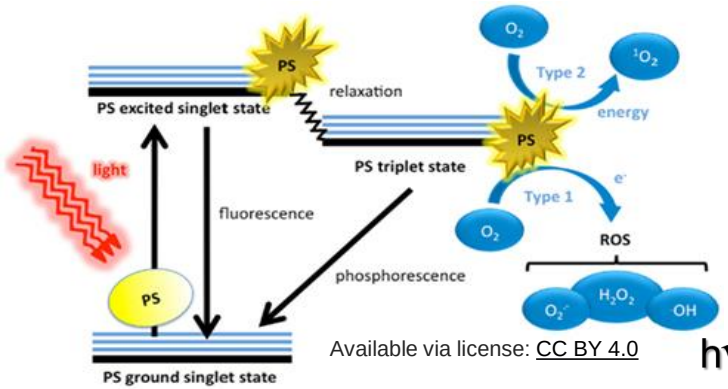
Biomedical Physics laboratory has all the necessary equipment for characterization of nanoparticles photophysical and physicochemical properties. Furthermore, our laboratory is fully equipped with bio-imaging devices and tools for *in vitro* and *in vivo* experimentation.

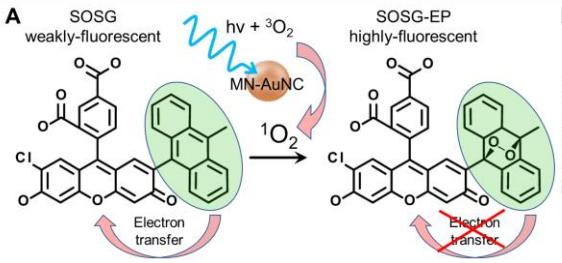
- Absorbtion, Steady-State and Time-Resolved Fluorescence Spectrometers
- Zeta Potential and Hydrodynamic Size Analyzer
- Nanoparticles Synthesis System
- Equipment for Cell Cultivation and Manipulations
- Flow Cytometer
- Nanotoxicity Evaluation Equipment
- Modular Confocal Laser Microscope System
- Atomic Force Microscope
- Optical Biopsy System with Monitoring Complex for Experimental Animals
- *In vivo* Confocal Imaging microscope

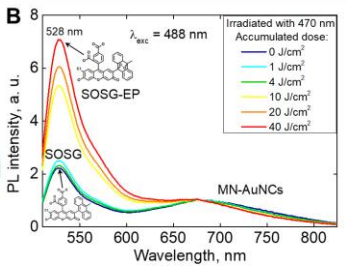




Multimodal cancer theranostics





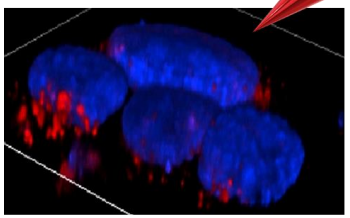


Generation of singlet oxygen was evaluated with “Singlet Oxygen Sensor Green”.

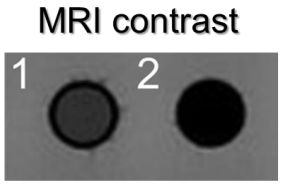
THERANOSTICS

Multimodal MN-AuNCs emit red photoluminescence in the range of biological transparency window, improving light penetration in tissue. Long-lived excited state also creates favorable conditions for generation of singlet oxygen/ROS.

Multimodal magnetic iron oxide nanoparticles decorated with photoluminescent gold nanoclusters (**MN-AuNCs**), exhibit both **MAGNETIC** and **OPTICAL** signals enabling **DUAL DIAGNOSTICS**. Moreover, they possess **THERAPEUTIC** properties (can generate singlet oxygen under visible light irradiation) suitable for **PHOTODYNAMIC THERAPY**.



MN-AuNCs in cancerous MCF-7 cells.



Clinical MR images show that these nanoparticles work as a T2 contrast agent.

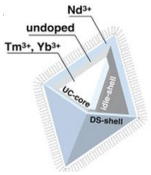
Nanoparticles were synthesized in collaboration with A. Jagminas research group from State Research Institute Center for Physical Sciences and Technology. Mikalauskaite A., Pleckaitis M., Jagminas A. et al., *RSC Adv.* (2022).

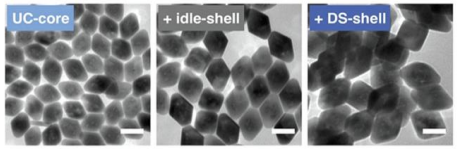
Pleckaitis M., Karabanovas V., Rotomskis R. et al., *Adv. Mater. Interfaces* (2023)

Decoupled cancer theranostics

Decoupled theranostics constitutes integrated therapeutic and diagnostic functions in a single drug-probe system, promising example of which are rare-earth doped nanoparticles (RENPs). We are exploring the possibility to optically decouple the diagnostic and therapeutic features of RENPs by using excitation wavelengths that can trigger these features individually. Also the NaGdF4 host was used as MRI contrast agent. This paradigm shift will bring RENPs closer to routine biomedical research and clinic, as true multi-functionality can be attained.

*d*NPs – design, synthesis of RENPs in collaboration with prof. F. Vetrone group (Canada).

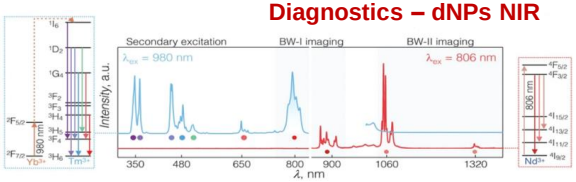




- On demand functionality
- Diagnostics without therapy
- Excitation wavelength specificity
- Multi-layered architecture

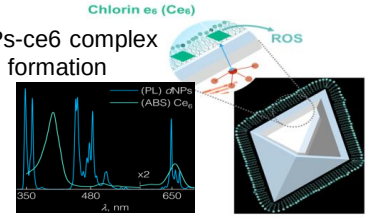
Therapy – dNPs visible

Diagnostics – dNPs NIR

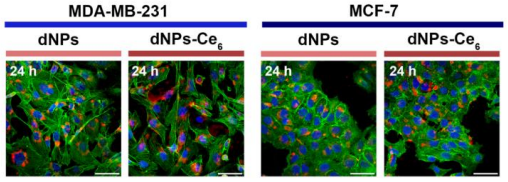


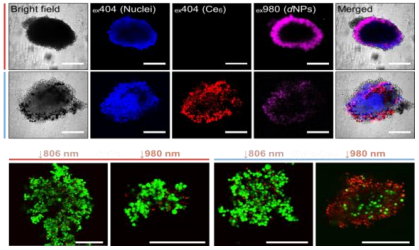
Inracellular accumulation of dNPs and dNPs-ce6 complexes

dNPs-ce6 complex formation

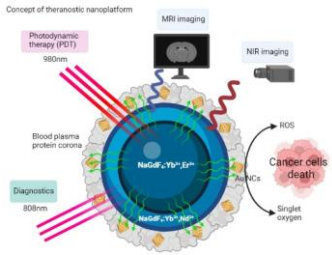


*d*NPs - photodynamic therapy (PDT) in cancer spheroids



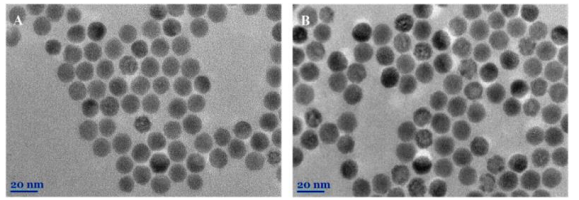


A. Skripka, V. Karabanovas et al., *Adv. Funct. Mater.* 2019.



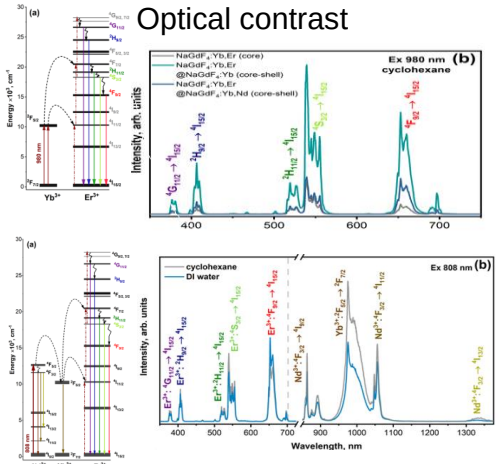
Universal multimodal platform able to perform NIR/MRI imaging and PDT

Collaboration with prof. A. Katelnikovas and dr. V. Klimkevicius groups (Lithuania)

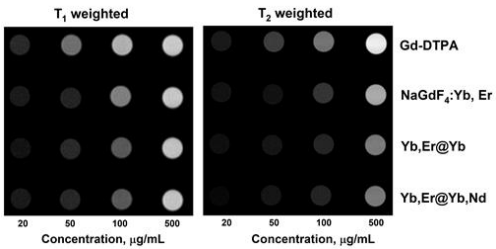


RENPs architecture: allow us to either visualize the particle by using MRI/NIR or initiate PDT using different NIR lasers

Optical contrast



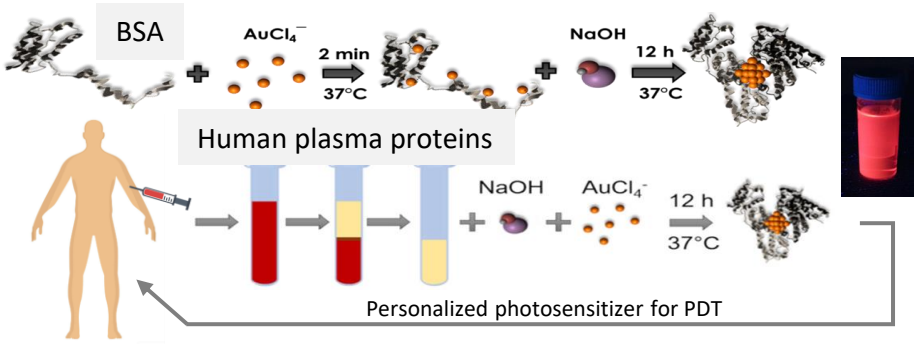
MRI contrast

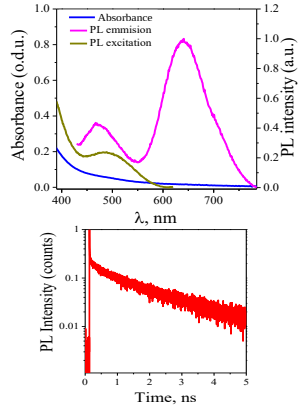


D. Baziulyte-Paulaviciene, V. Karabanovas et al., *Belstein,* 2017.
V. Klimkevicius, V. Karabanovas et al., *Journal of Materials Science B*, 2021.

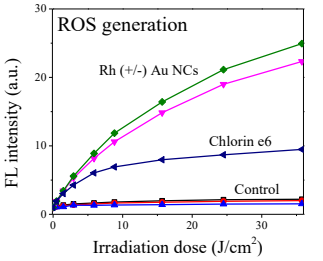
Plasma proteins decorated with gold nanoclusters for personalized PDT

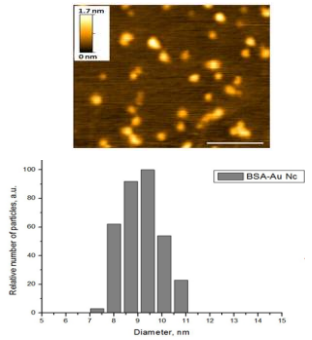
Synthesis of Protein decorated Au NCs





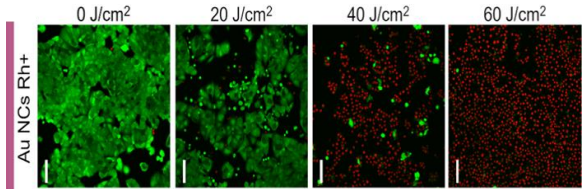
Characterisation

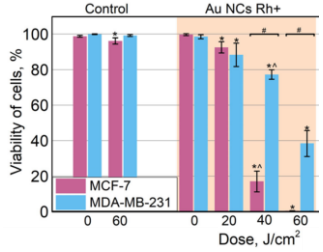




Poderys V. et al. Journal of photochem. and photobiology B: Biology. 2020

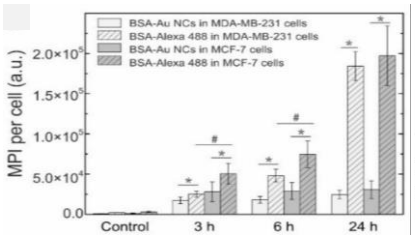
PDT in cells

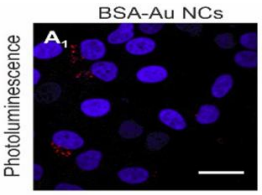


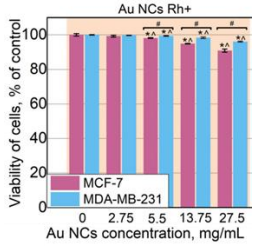


Jarockyte G. et al., Cancers, 2022

Cytotoxicity, accumulation in cells







Proteins decorated with gold nanoclusters can be synthesized using plasma proteins obtained from a patient and can be used for developing personalized PDT and theranostics agents. Investigation of these nanoparticles in our laboratory includes :

- Synthesis of proteins decorated with gold nanoclusters. BSA and human blood plasma are used for synthesis. Investigation of Human blood plasma proteins decorated with gold nanoclusters as personalized theranostic agents.
- Characterization of protein-gold nanoclusters using steady state and kinetic spectroscopy, dynamic light scattering, zeta potential, atomic force microscopy and other techniques. Investigation of singlet oxygen and ROS generation using fluorescent probes.
- Cytotoxicity and accumulation dynamics of nanoclusters in various cell lines.
- Investigation of Plasma proteins decorated with gold nanoclusters as potential personalized photosensitizers for PDT.
- Use of plasma proteins decorated with gold nanoclusters for personalized theranostics and multimodal nanoparticles

Radiolabeling of Gold nanoparticles for *in vivo* Imaging

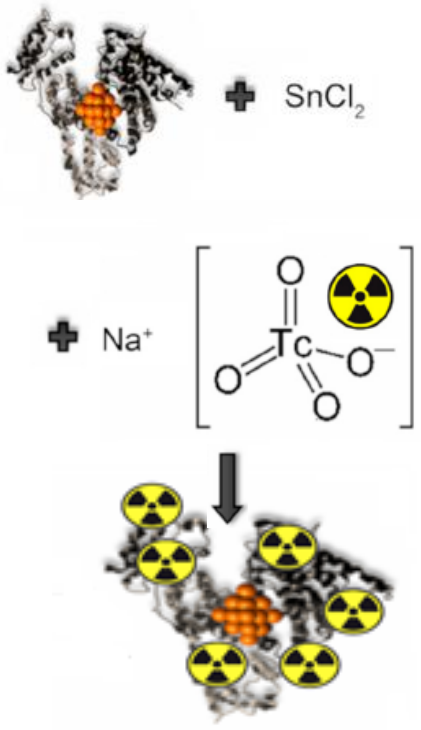
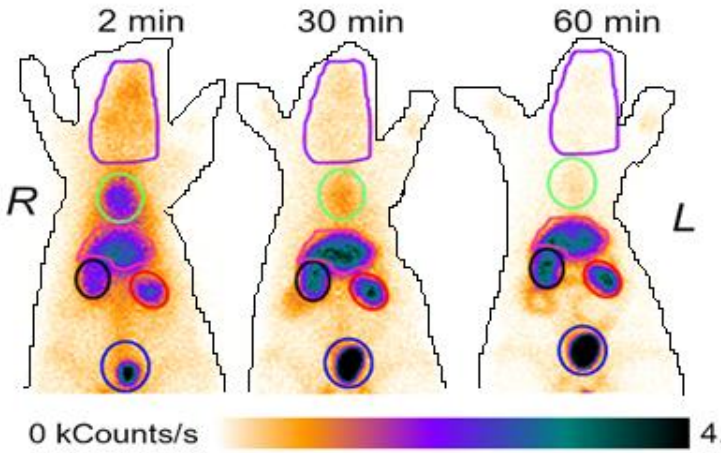
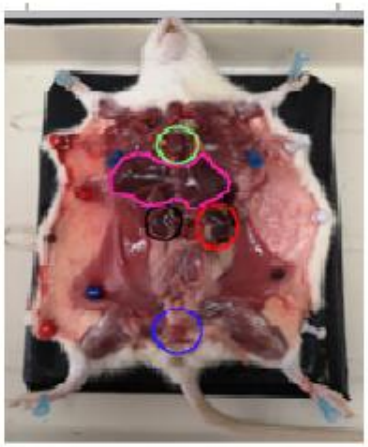
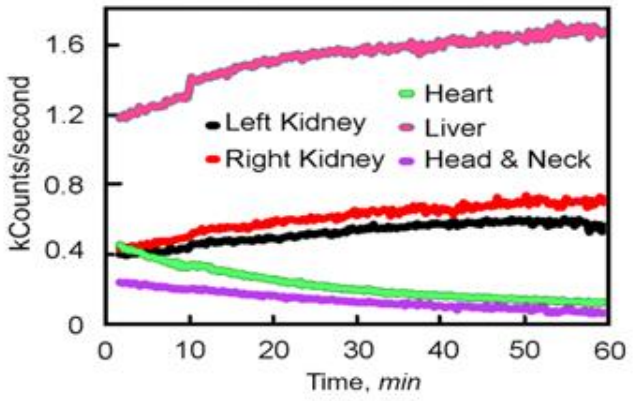


Diagram for the radiolabeling of gold nanoclusters with technetium-99m



Dynamic images showing uptake and the distribution of the gold-protein nanoparticles in different organs of Wistar rat animal model. Regions of interest are drawn for the investigation of the heart, the liver and both kidneys.



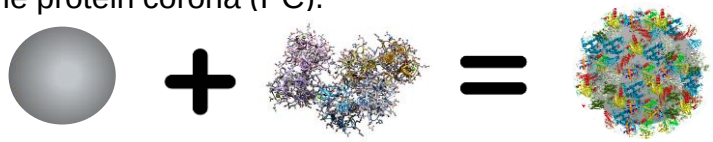


Jarockyte G., Stasys M., Rotomskis R. et al. *Nanomaterials* (2022)

Enhancement of nanoparticles' uptake by cancer cells. Protein corona approach

What is protein corona?

When a nanoparticle (NP) is in the blood or cell growth medium, proteins adsorb onto the NP surface, creating an additional layer called the protein corona (PC).



Nanoparticle | Proteins | Nanoparticle-protein corona complex

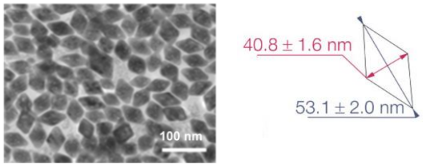
Why PC studies are important?

Promising NPs' diagnostic and therapeutic results relies on great uptake of NPs by cancer cells. To achieve this, NPs have to be coated with appropriate surface coatings that "attract" specific protein profile on its surface. The cell itself do not see the NPs, the cells see the upper layer of the NP – the PC, or the NP-PC complex, in general. Also, PC formation around NP is unavoidable and can result in rapid clearance by mononuclear phagocytic system. Thus, appropriate surface coatings of NPs can:
1) enhance cellular uptake of NPs by attracting proper proteins on the surface for better cellular internalization; 2) extend blood half-life circulation time of NPs.

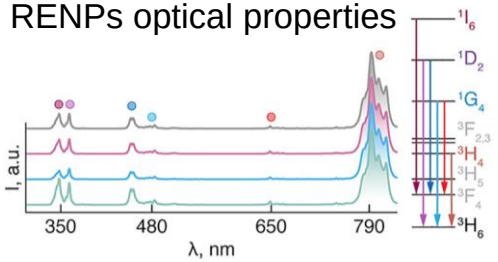
What type of NPs was used for the studies?

Diamond-like shape various coatings as citrate, phospholipids, SiO_2 bearing $LiYF_4$: Yb^{3+}, Tm^{3+} rare-earth doped NPs (RENPs) that exhibit emission in the biological transparency window (700-950 nm).

Size and morphology of RENPs

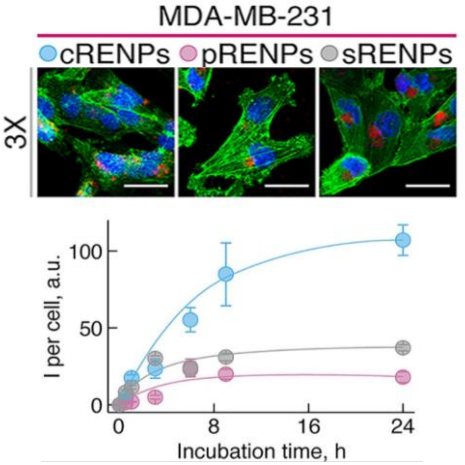


RENPs optical properties



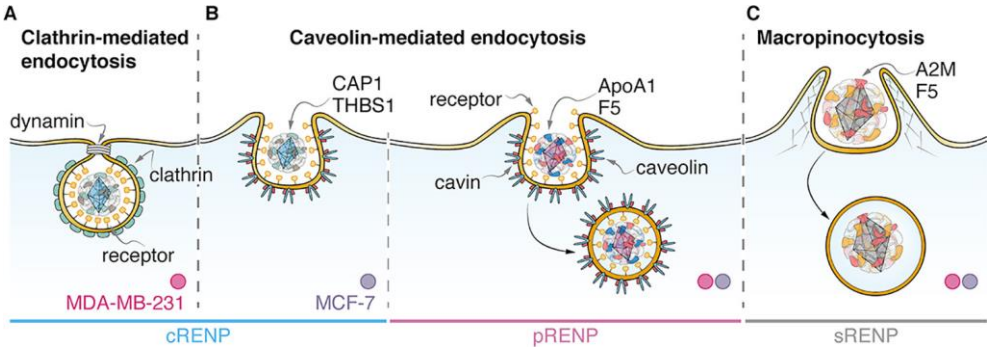
RENPs have strong emission at biological optical transparency window (790 nm)

Cellular uptake dynamics



Different uptake dynamics of RENPs regarding the RENPs' surface.

Surface-determined cellular internalization of RENPs

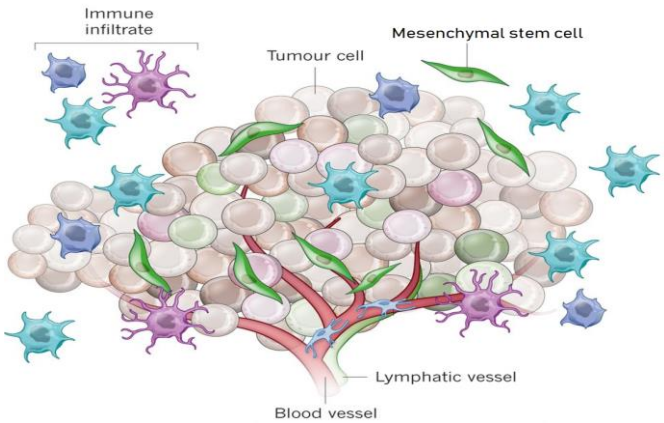


Endocytic routes of different coating bearing RENPs depend on: 1) protein profile of the PC formed around the each RENP; 2) cell line surface proteomics; 3) RENPs surface modification.

Voronovic E., et al. *ACS Applied Materials & Interfaces* (2021)

MSCs as cellular vehicles for targeted delivery of theranostic agents

Tumor tropism



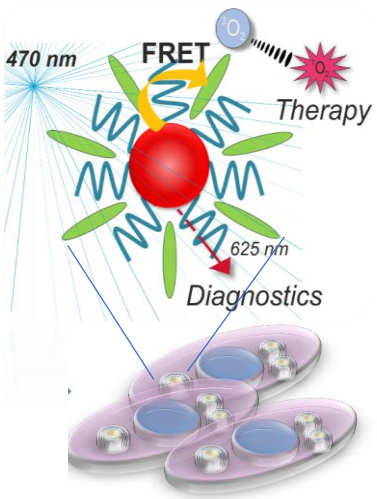
Junttila & de Sauvage, 2013

Mesenchymal stem cells have specific receptors for binding chemokines released by tumors. After the chemokine signaling mesenchymal stem cells migrate towards the chemokine gradient (tumor).

Quantum dot

Photosensitizer

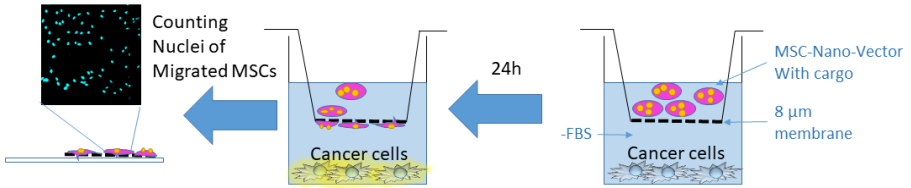
- **Theranostic agents** are light-activated materials. They are non-toxic and bio-friendly unless light of a specified wavelength is used and ROS generation is activated.
- We isolated human **mesenchymal stem cells** from human bone marrow, skin and menstrual blood (ethical permit no. 158200-18/6-1036-548).

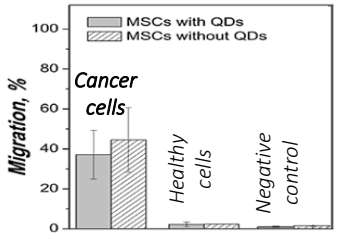


Mesenchymal stem cells

Theranostic agent

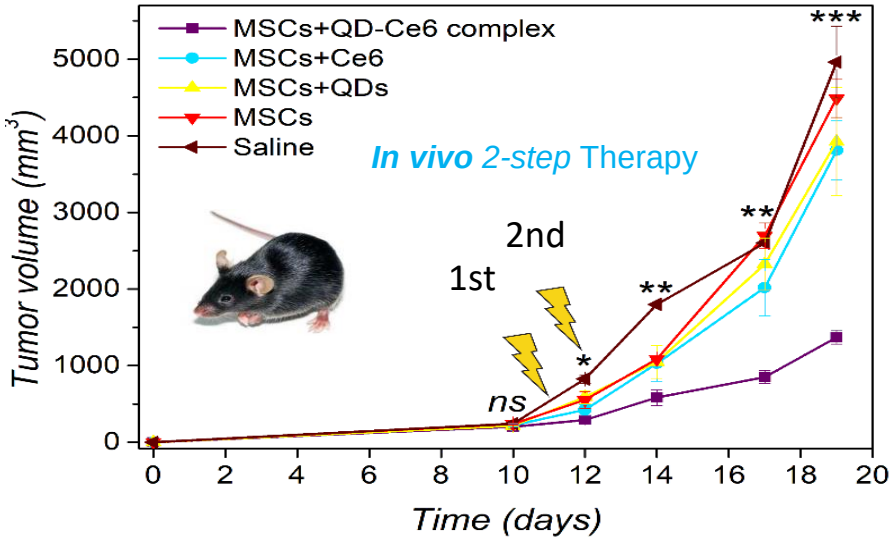
Tumor-tropic migration of MSCs with Theranostic agent





Tumor-tropic migration is assessed using *Transwell* migration assay. MSCs uploaded with theranostic cargo tend to migrate towards cancer cells, but not healthy cells or culture medium DMEM (negative control), thus ensuring **targeted delivery.**

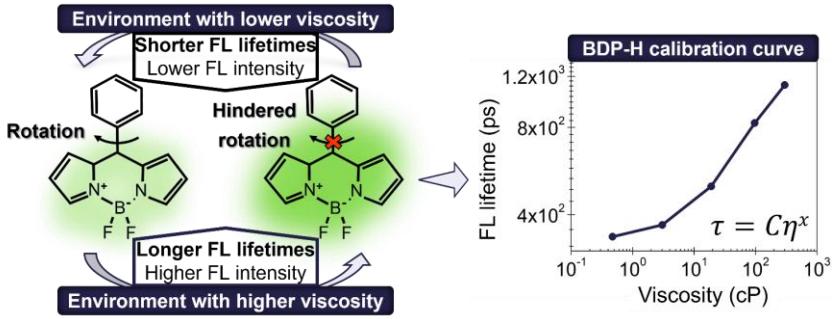
Dapkute D., Steponkiene S. et al. *International Journal of Nanomedicine* (2017)



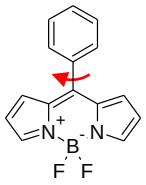
Dapkute D., Karabanovas V. et al. *ACS Applied Materials & Interfaces* (2021)

Molecular rotors and microviscosity

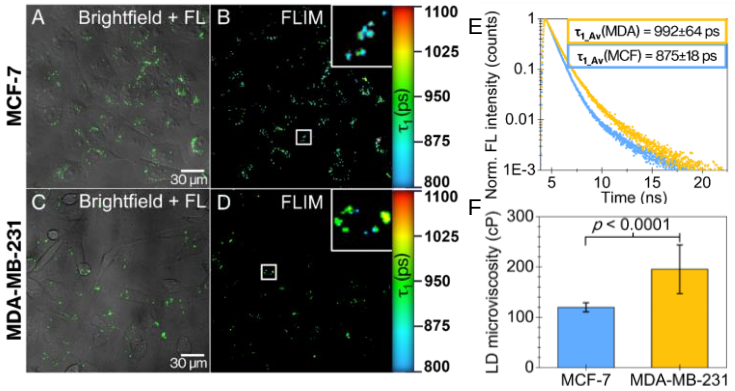
- Conventional mechanical methods are inapplicable for measuring viscosity at the microscopic level.
- Introducing a phenyl ring at the *meso* position of boron-dipyrrin or BODIPY fluorophore renders the molecule sensitive to changes in microviscosity.
- The mechanism of molecular rotors or viscosity-sensitive fluorophores is based on the competition between fluorescence and non-radiative relaxation. Together with fluorescence lifetime imaging microscopy (FLIM), molecular rotors enable microviscosity imaging at the subcellular level.



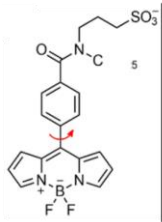
Monitoring microviscosity in live cancer cells



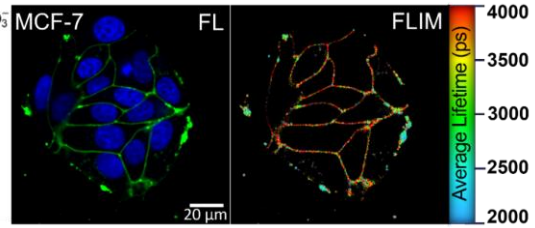
BDP-H



BDP-H molecular rotor accumulates in lipid droplets of human breast cancer cells. BDP-H with FLIM revealed that lipid droplet microviscosity in highly-aggressive MDA-MB-231 cells is significantly elevated as compared to MCF-7 cells.



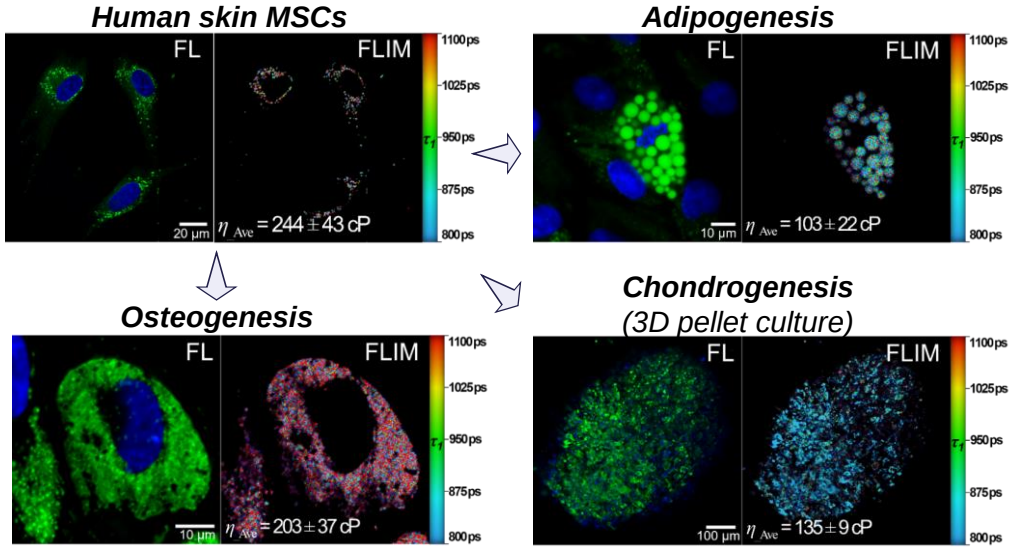
BODIPY-PM



Jurgutis D. et al., *Int. J. Mol. Sci.* (2022)

Another molecular rotor – BODIPY-PM, can be applied for monitoring microviscosity in plasma membranes.

Measuring microviscosity in differentiated MSCs



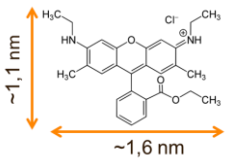
Measuring microviscosity of lipid droplets in differentiated human skin mesenchymal stem cells (MSCs) can provide a better characterisation of adipogenic, osteogenic, and chondrogenic differentiations at the organelle level, thereby enhancing our understanding of the underlying causes of disorders associated with these cell types.

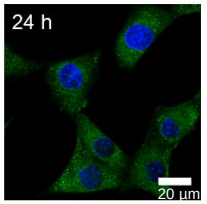
3D cell cultures as a model for accumulation and distribution studies

Potential drug | Cell monolayers | 3D spheroid cell cultures | Mathematical description | Comparison to *in vivo*

Firstly, potential drugs are investigated *in vitro*

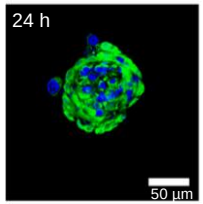
Small molecules





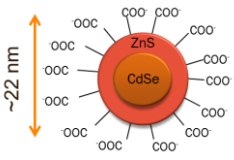
Passive accumulation (diffusion)

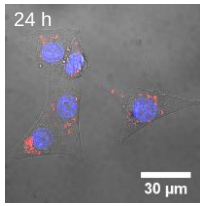
3D cell cultures fill the gap between cell monolayers and laboratory animals



Passive accumulation (diffusion)

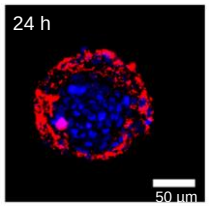
Nanoparticles





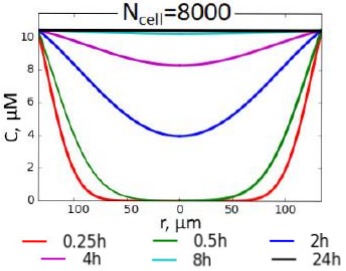
Active accumulation (endocytosis)

Confocal microscopy allows 3D sectioning though spheroid

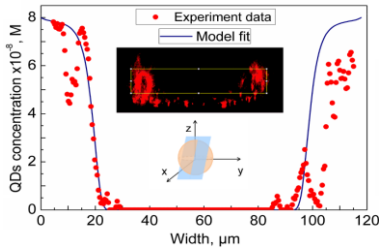


Active accumulation (endocytosis)

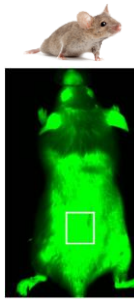
Better understanding of processes

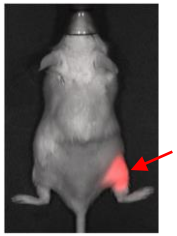


Astrauskas R., Jarockyte G. et al., *Nonlinear Anal.: Model. Control.* (2019)



Jarockyte G. et al., *Biochim Biophys Acta Gen Subj*. (2018).





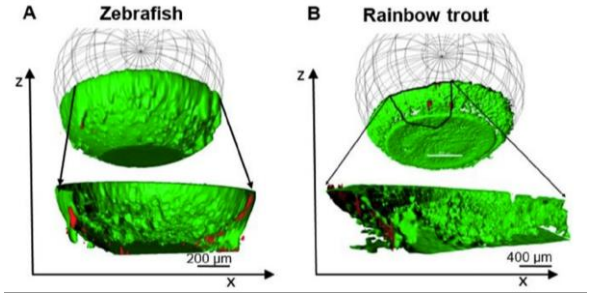
- 3D spheroids cell culture – self-assembled clusters of cell colonies cultured in environments where cell-cell interactions dominate. 3D spheroid cell cultures mimic the environment of *in vivo* avascular tumors: they possess the diffusion gradient of drugs, oxygen, nutrients, and waste.
- Cellular spheroids could be used as model system for various researches, such as nanoparticles or fluorescent dyes penetration modelling studies, investigation of nanoparticles theranostic potential.
- In Biomedical Physics laboratory, 3D spheroid cell cultures are formed using the hanging drop and forced floating methods. Diameters of spheroids depend on the cell line, initial number of cells and growth time.

Toxicological Assessment of Engineered Nanoparticles

Toxicological research is also a crucial component of the Biomedical Physics Laboratory's work. In this facility, research involving *Daphnia magna, z*ebra fish, rainbow trout, their larvae, embryos are done.

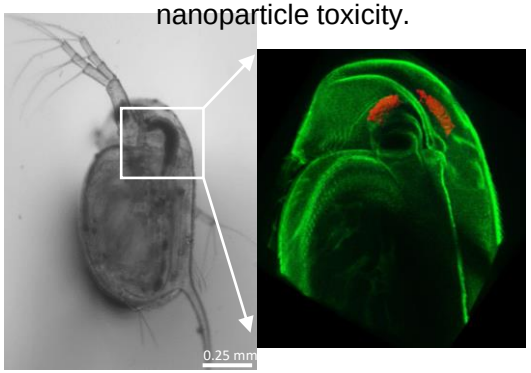
Visualization of Embryos exposed to quantum dots using confocal microscopy:

Zebra fish Rainbow trout

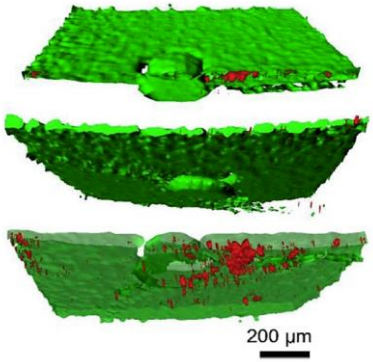


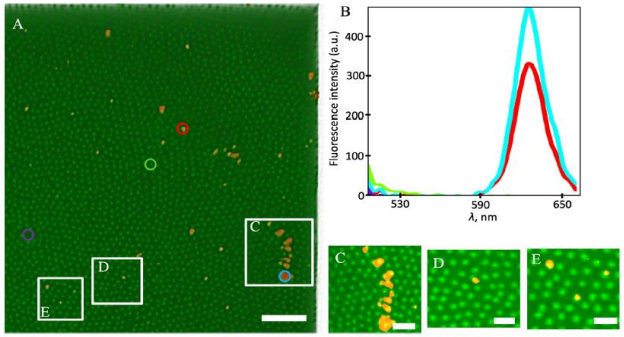
Rotomskis R., Karabanovas V. et al., *Science of the Total Environment (*2018)

Utilizing *Daphnia Magna* can provide valuable insights into the mechanisms underlying nanoparticle toxicity.



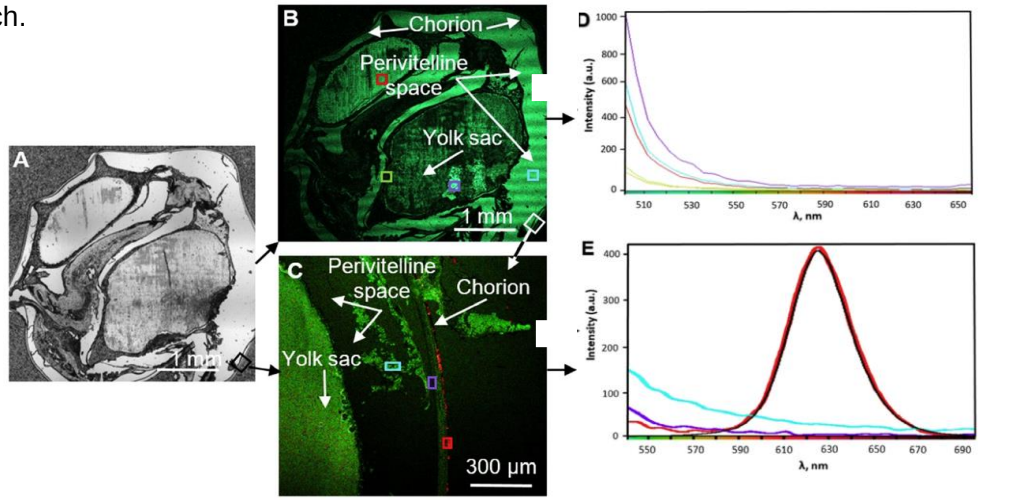
Confocal microscopy enables the creation of 3D models while keeping the test organisms alive. This approach allows us to uncover intricate details about the toxic effects and pathways of various nanomaterials, including where and how nanoparticles can aggregate inside or outside the organisms.





Jurgelėnė Ž., Karabanovas V., Rotomskis R. et al., *Science of the Total Environment* (2021)

Confocal imaging is not the sole method for gaining comprehensive insights into nanoparticle toxicity. Histological images combined with photoluminescence spectra analysis represent an alternative approach.



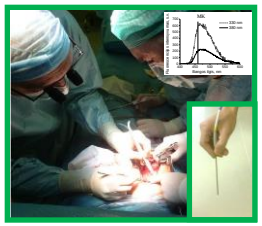
Rotomskis R., Karabanovas V. et al., *Science of the Total Environment* (2018)

Clinical and preclinical studies

Optical biopsy - endogenously present molecules or externally introduced contrast agents possesses distinctive optical properties under different excitation conditions. Therefore, non-invasive differentiation of tissues and/or visualization can be performed.

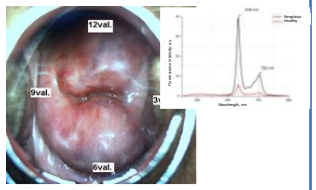
Fluorescence

Differentiation of heart conduction system during surgery procedure



Venius J., Rotomskis R. et al. *Journal of Biomedical Optics* (2011)

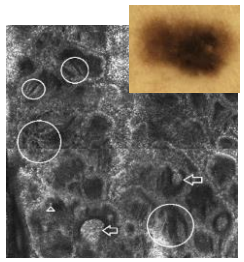
Early cervical pre-malignancy identification



Vansevičiūtė R., Venius J., Rotomskis R. et al. *BMC Womens Health* (2015)

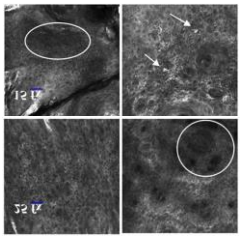
Reflectance Confocal Microscopy *in vivo*

Melanoma detection



Vaišnorienė I., Rotomskis R., Venius J. et al. *Medicina* (2014)

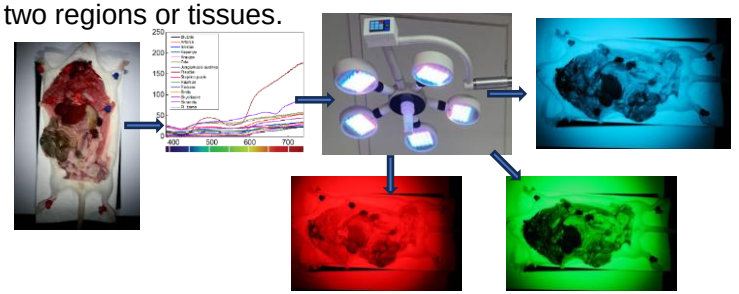
Radiation-Induced Acute Radiodermatitis detection



Kišonas J., Venius J., Rotomskis R. et al. *Diagnostics* (2021)

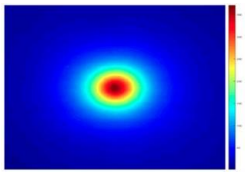
Smart illumination solutions

Depending on tissues of interest during the surgery, specific illumination, based on tissues optical properties, are formed to create the best possible contrast between two regions or tissues.



Adaptive brachytherapy – during focal brachytherapy of prostate cancer partial irradiation of the prostate with high dose gradients is performed. High gradient ensures that cancerous tissue receives critical dose, and it does not spread to the healthy tissues or critical structures. However, if during treatment procedure some deviations from planned treatment will occur, there is a risk that dose will not cover the whole tumour. Real time dose monitoring solution has been developed to overcome this situation.

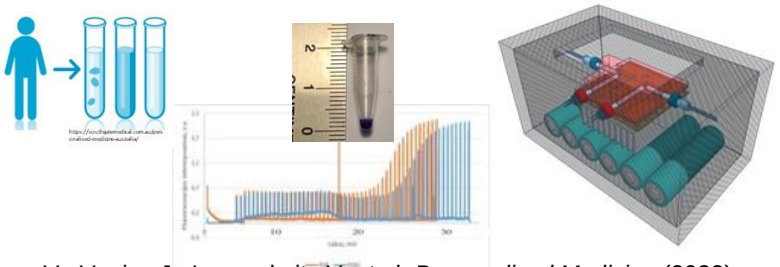
Technique developed by our team comprises new type microdosimeters and a software for precise dose verification.



Real time imaging of radioactive source position and dose monitoring to verify correct dose distribution.

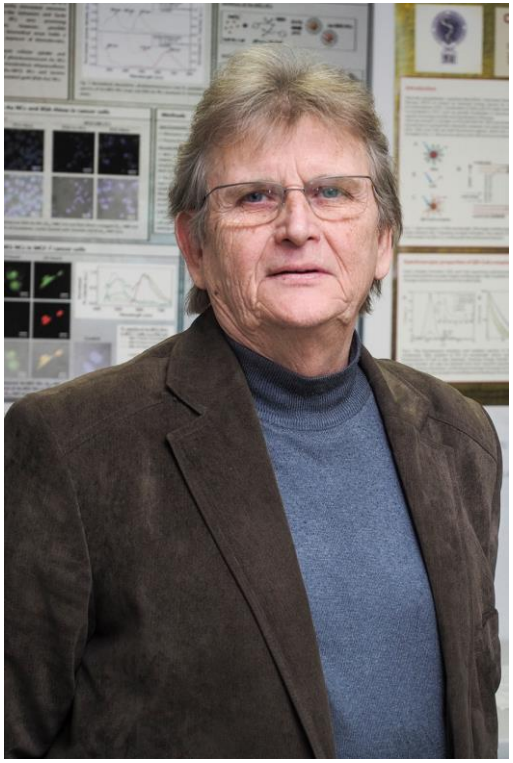
Patent No. EP4186562A1

Point of care – blood direct PCR for personalised drug prescription.
Technique developed by our team connects a new one-step and one-tube strategy of novel biomarker identification with a dedicated and newly developed solution.
The principle of the method is based on fast identification of genetic variant which determines the activity of antiplatelet drugs directly from 0,2 µL of blood sample.
European patent pending.



Tatarunas V., Venius J., Lesauskaite V. et al. *Personalized Medicine* (2022)

Contacts



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