



HABILITATION DOMAIN: BIOLOGY

HABILITATION THESIS

ABSTRACT

**Bioindicators and biomarkers
with applications in Ecology and Biomedical sciences**

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Abstract

The objective of the habilitation thesis entitled “*Bioindicators and biomarkers with applications in Ecology and Biomedical sciences*”, developed in the field of Biology, is to analyze and integrate the teaching and scientific achievements carried out during the postdoctoral period. The thesis reflects the evolution of contributions in the fields of ecology, histology, and molecular histology, as well as their impact on modern research directions and biomedical applications. In addition to reviewing past achievements, the thesis outlines strategic directions for professional development in the next stage of the academic career, in accordance with the recommendations of CNATDCU and the applicable legislation.

The academic career began with the attainment of the Doctorate in Biology, following the public defense of the doctoral thesis “*Eco-morphological Research on Certain Plecoptera and Ephemeroptera Larvae from the Tributaries of Lake Bicz*” at the University of Bucharest, under the supervision of Prof. Maria Caloianu¹. The title of Doctor in Biology was officially conferred by the Order of the Minister of Education and Research no. 5237 on November 5, 2004.

The habilitation thesis is structured into two main sections. The Section I – **Scientific and Teaching Achievements** – presents the postdoctoral contributions in ecology, histology, and molecular histology, including research activities, scientific output, results obtained from projects, and teaching experience gained at the university level. This section is structured into two main parts: Bioindicators and biomarkers with applications in Ecology and Environmental protection and Biomarkers with applications in Biomedical sciences. The first part of Section I addresses bioindicators and biomarkers with applications in Ecology and Environmental protection, emphasizing their complementary roles in the assessment and long-term monitoring of freshwater ecosystem quality. The research is grounded in a holistic reservoir–catchment perspective, which highlights the connectivity between rivers, reservoirs, and their surrounding landscapes. Particular attention is given to the use of aquatic bioindicators, especially benthic macroinvertebrates, for evaluating running water quality, river–lake ecological succession, and ecosystem responses to anthropogenic pressures. By integrating traditional ecological assessment methods with histological and molecular biomarkers, this part provides an advanced

and sensitive framework for assessing pollutantsensitivity and environmental stress in aquatic ecosystems. The second part of Section I focuses on biomarkers with applications in Biomedical sciences, with particular emphasis on oncology, molecular histology, and proteomics. This section addresses the limitations of conventional diagnostic approaches and highlights the role of Biology and modern omics technologies, such as Proteomics, in the identification and characterization of novel protein biomarkers. Special attention is given to the *in situ* detection of biomarkers using Bright-Field (BF) and fluorescence microscopy (FLUO), as well as to the significance of post-translational modifications (PTMs) and the multifunctional roles of biomarkers in cancer development and progression. The research adopts an integrative and holistic perspective on tumorigenesis, contributing to a deeper understanding of breast cancer (BC) biology and to the advancement of translational biomedical research.

The Section II – **Development of Teaching and Scientific Career** – details future directions, with a focus on strengthening research in the field of bioindicators and molecular biomarkers, expanding national and international collaborations, developing laboratory infrastructure, increasing efforts to secure research funding, and promoting internationalization and modernization of the teaching process.

The integration of these two sections highlights both the achievements accomplished and the vision for future academic development, essential for consolidating a competitive professional profile adapted to the current requirements in the field of biological sciences. The results obtained demonstrate the ability to build and lead complex research teams composed of specialists, doctoral students, master's students, and undergraduates, as well as the capacity to successfully secure funding for original research projects.

The teaching activity has been supported by integrating our research results into the programs and specializations of the Faculty of Biology at "Alexandru Ioan Cuza" University of Iași. The topics and skills developed in the fields of bioindicators, biomarkers, histology, developmental biology, and applied ecology are reflected in the courses taught: **Animal Histology** (Biology I and Biochemistry I), **Human Anatomy and Hygiene / Human Anatomy and Physiology** (Faculty of Biology: Biology I; Faculty of Physics I and III: Physics, Medical Physics, and Biophysics; Faculty of Chemistry I: Chemistry, Medical Chemistry, Biochemistry), **Developmental Biology** (Biology II, Biochemistry II), **Animal Ecomorphology** (Ecology and Environmental Protection I), **Bioindicators of Environmental Quality** (Master's in Environmental Counseling I), **Xenobiotic Cycle in Nature** (Master's in Environmental Counseling II), **Immunohistochemistry** (Master's in Medical Laboratory I),

Molecular Biomarkers (Master's in Molecular Genetics II), and **Microscopy** (Biology III). This integration ensures a solid theoretical and practical foundation, preparing undergraduate, master's, and doctoral students for engagement in modern research and the application of laboratory techniques.

We have developed, in collaboration, significant international partnerships, including participation in three European projects for the implementation of the European master's program "*Ecological Management of Catchments in Europe*" (ECOCATCH), in Sweden and at partner European universities, under the coordination of Uppsala University. In addition, the modernization of the **Laboratory of Animal histology and Confocal microscopy** at the Faculty of Biology has provided students with essential practical skills for animal tissue processing and the preparation of permanent microscopic slides. Through this upgraded framework, numerous students have acquired strong technical abilities and successfully integrated into medical, histology, or pathological anatomy laboratories, demonstrating valuable practical competencies and benefiting from training that paved the way for stable and long-term professional careers. This laboratory represents not only an investment in teaching infrastructure but also a direct contribution to students' professional development and the growth of human capital in the biomedical field.

I have been director of three research projects (Assessment of polluosensitivity of some bioindicators of water quality using morpho-histological characteristics; Extension of the laboratory of Aquatic ecology Potoci for assessing the mountain reservoirs pollution induced by floating trout farms, according to the Water Framework Directive 2000/60/CE; Development of the infrastructure of the **Laboratory of Bioindicators of water quality ACVAPUR Potoci** to assess the polluosensitivity of aquatic organisms) with a total budget of 533.500 € and member or external collaborator in other national research projects. Through the ACVAPUR Laboratory, established at the Potoci Biological Station, specialized compartments were created and developed for analyzing bioindicators at different levels of the trophic network. This infrastructure enabled competent, interdisciplinary, and integrated evaluation of the water quality of Lake Bicz and its tributaries, while also providing a model applicable to other reservoirs in the context of trout aquaculture in floating salmon farms, preserving the natural self-purification capacity of lacustrine ecosystems. The laboratory's activities supported the completion of a significant number of doctoral theses and facilitated the organization of two editions of the Summer School of Limno-ecology, in partnership with the University of Konstanz (Germany), thus contributing to the training of specialists in water quality assessment

based on bioindicators. Overall, the activities carried out at ACVAPUR Laboratory have represented an important hub for training and applied research. I have also supervised 75 bachelor's and master's theses in histology and ecology, consolidating the role of these two laboratories as platforms for advanced professional training for undergraduate, master's, and doctoral students.

Over the past eight years, research and teaching activities have focused on molecular histology, proteomics, and protein biomarkers, with the aim of identifying new biomarkers for BC. In this context, I have collaborated with the Biochemistry and Proteomics Laboratories at Clarkson University (USA), coordinated by Prof. Dr. Costel Darie, resulting in 24 articles as the lead author. The book entitled "*Bioindicators of running water quality*" was awarded by Romanian Academy ("Grigore Antipa" Award) and by Academy of Romanian Scientists ("Emil Pop" Award). The book chapter published by Springer Nature Switzerland in 2019 is included into the book "*Advancements of Mass Spectrometry in Biomedical Research*"⁴ (Woods & Darie eds.) that has been awarded "Daniel Danielopolu" Prize at Section of Medical Sciences of Romanian Academy in 2021.

The national and international collaboration will be expanded through national and international projects, in which the Laboratory of Animal histology and Confocal microscopy will contribute to the immunohistochemical (IHC) and immunofluorescent (IF) detection of biomarkers, in collaboration with the Department of Morphofunctional Sciences and the Advanced Research and Development Center in Experimental Medicine "Prof. Ostin C. Mungiu" / CEMEX – "Grigore T. Popa" University of Medicine and Pharmacy, Iași, as well as with the Regional Oncology Institute of Iași. These projects and collaborations will provide a solid framework for doctoral students, supporting their specialization and deepening of knowledge in molecular histology and protein biomarkers.

A key objective of professional development is the continuous participation in advanced training programs, through international courses, trainings, and workshops dedicated to the most modern technologies used in biomedical research, such as proteomics, IHC, advanced IF-based microscopy for multiplex detection, single-cell analysis, and bioinformatics applications for multi-omics data interpretation. Research results will be presented at national and international scientific events and published in specialized journals.

Simultaneously, the implementation of Molecular ecology can open new perspectives in environmental quality assessment, both through bioindicators and biomarkers for the diagnosis and monitoring of ecosystems.

The Laboratory of Animal histology and Confocal microscopy will continue to provide support and assistance to pre-university students, facilitating their preparation for participation in the National Biology Olympiad and other specific competitions. This engagement contributes to attracting young people to the specializations offered by the Faculty of Biology and stimulates the interest in the study of life sciences. At the same time, organizing new photomicrography exhibitions will help popularize research and teaching activities at both the local and national levels, strengthening the connection between the laboratory, the academic environment, and the broader public.

Acknowledgments

I dedicate this habilitation thesis, particularly the part resulting from research on bioindicators of running water quality, to Professor *Emeritus* dr. Ionel Miron, full member of the Academy of Romanian Scientists, my first mentor, on the occasion of his remarkable 90th birthday.

SECTION I. SCIENTIFIC AND TEACHING ACHIEVEMENTS

1.1. Bioindicators and biomarkers with applications in Ecology and Environmental protection

1.1.1. Research motivation

This subchapter highlights the need to develop sensitive and integrated biological tools for assessing the impact of anthropogenic pressures on aquatic ecosystems, given the limitations of traditional physico-chemical methods. Bioindicators- and biomarkers-based methods are proposed as effective approaches for environmental quality monitoring, as they can capture cumulative, synergistic, and sublethal effects of pollution, supported by modern ecotoxicological and multi-omics methodologies. The research presented builds on the foundations of my doctoral thesis and subsequent postdoctoral activity, with an emphasis on aquatic insects as bioindicators, ecomorphological biomarkers, and the development of an integrative catchment–lacustrine ecosystem framework applied to the Bicz Reservoir case.

I also emphasize the essential role of training specialists in applied ecology, biomonitoring, and molecular ecology, particularly in the context of accelerating environmental degradation and the need to align Romanian research with international standards. By integrating morphological, molecular, and proteomic analyses, we propose an interdisciplinary approach that contributes both to advancing fundamental ecological knowledge and to the development of effective strategies for ecological management and environmental protection.

1.1.2. Bioindicators and biomarkers: their role in the assessment and monitoring of environmental quality

In this section, we discuss the role of bioindicators and biomarkers as complementary tools in the assessment and monitoring of environmental quality, emphasizing their advantages over conventional physico-chemical methods, which often provide only fragmentary and static information. We highlight the paradigm shift from contaminant-based monitoring towards effect-based approaches, in which bioindicators reflect changes at population and community levels, while biomarkers reveal early biological alterations at molecular, cellular, tissue, or physiological levels. I outline the conceptual evolution and definitions of both terms, stressing their interdependence and their relevance for integrated ecological risk assessment. Particular

attention is given to the ecological value of sensitive aquatic organisms, especially benthic macroinvertebrates, as reliable bioindicators of freshwater quality. I also address the strengths and limitations of different classes of biomarkers and underscore the importance of multi-level and multi-omic approaches for early detection of environmental stress.

Overall, I argue that the combined use of bioindicators and biomarkers provides a robust, sensitive, and ecologically meaningful framework for modern environmental monitoring and management.

1.1.3. The holistic concept: lake-catchment

In this subchapter, we adopt a holistic lake–catchment perspective, emphasizing that lakes, particularly reservoirs, are integral components of natural capital and provide essential ecosystem services. I highlight that the ecological status of a lake is directly linked to the quality of its tributaries and to the physical, chemical, biological, and land-use characteristics of the entire catchment area. Building on the principles of modern limnology and the requirements of the EU Water Framework Directive (2000/60/EC), we focus on the assessment of ecological quality using biological indicators, especially benthic macroinvertebrates, as reliable integrators of environmental pressures.

This research, initiated during my doctoral studies and extended over the subsequent decade, applies this integrated framework to the Bicaz Reservoir and its tributaries, demonstrating how upstream anthropogenic pressures, hydrological variability, nutrient inputs, and sediment dynamics shape lacustrine ecosystem functioning. Overall, I argue that sustainable water resource management requires an integrated, basin-wide monitoring strategy in which bioindicator-based ecological assessment plays a central role.

1.1.4. Use of biomarkers for the assessment of running water quality

In this section, I present the use of biological methods based on bioindicators for the assessment of running water quality, emphasizing their advantages over conventional physico-chemical approaches in terms of cost-efficiency and ecological relevance. I focus on benthic macroinvertebrates, particularly *Ephemeroptera*, *Plecoptera*, and *Trichoptera* (EPT), as key bioindicators widely used in biotic indices across Europe and globally. I describe the adaptation and application of international biomonitoring methods in Romania, highlighting the IBGN index as the most suitable tool for evaluating the ecological status of streams within the Bicaz Reservoir catchment. Our expertise in applying this method was strengthened through

specialized training in France and further developed through participation in several European research projects, including the ECOCATCH programme.

These activities contributed to the advancement of biological monitoring methodologies, the integration of Romanian research into international frameworks, and the training of young researchers in modern limnological and ecological assessment techniques. Overall, this work supports the role of bioindicator-based approaches as essential components of sustainable management and protection of running water ecosystems.

1.1.5. River-lake ecological succession

In this section, I address river–lake ecological succession as a key process underlying the structural and functional transformation of aquatic ecosystems following river impoundment. Building on classical limnological frameworks, we adopt an integrated methodological approach that examines ecosystem changes at idiographic, cenographic, and holistic (ecosystem) levels. I discuss how dam construction induces a rapid and profound secondary successional process, marked by abrupt shifts in abiotic conditions, such as temperature regime, oxygen availability, hydrostatic pressure, and light penetration, which in turn drive major biotic restructuring. Using the Bicaz Reservoir on the Bistrița River as a case study, we describe the transition from rheophilic to stagnophilic communities and the substantial loss of benthic macroinvertebrate diversity, with only a small fraction of the original riverine fauna persisting.

These findings indicate that river–lake succession is faster and more pronounced in systems initially dominated by rheophilic species, highlighting the ecological consequences of river regulation and the importance of integrated ecological assessment in reservoir management.

1.1.6. Materials and methods

In this section, I present the materials and methods we employed to assess the water quality of the Bicaz Reservoir catchment. Our approach was based on multiyear studies using benthic macroinvertebrates as bioindicators, in accordance with modern ecological biomonitoring principles. I adapted internationally standardized methods, including the French IBGN and the Danish DSFI, to the specific hydromorphological and ecological conditions of local rheophilic streams, and proposed a Romanian biotic index (RIBI) to further develop national assessment tools.

Sampling protocols were designed to account for microhabitat diversity, substrate types, and flow regimes, ensuring representative benthic macroinvertebrate collections. Taxonomic identifications were mostly conducted at the family level, with genus- or species-level determinations applied selectively for specific objectives. Faunal data were synthesized into biotic scores, such as the IBGN, allowing classification of ecological quality from very good to very poor.

To support these studies, I coordinated and directed three national research projects (2005–2009), which enabled the development of two interconnected laboratories: the Laboratory of Animal histology and Confocal microscopy and the Laboratory of Bioindicators of water quality ACVAPUR Potoci. These facilities, along with a fully equipped research boat, advanced analytical instruments, and field equipment, allowed integration of field sampling with laboratory analyses, including SEM and TEM preparations.

The combination of standardized bioindicator methods, adapted to local conditions, and robust research infrastructure ensured rigorous, comparable, and ecologically relevant assessments of water quality. This work provided a foundation for national monitoring strategies, strengthened scientific capacity, and contributed to sustainable management of aquatic ecosystems in Romania.

1.1.7. Results and discussion

Through the three research projects I coordinated, we established the methodological, logistical, and human resources needed to support six doctoral theses completed by our team members (supervisor Prof. dr. Ionel Miron). The outcomes of our work were synthesized in two co-authored volumes: *Bioindicators of Running Water Quality* (2008), awarded by the Romanian Academy, and *Ecological Succession: Bistrița River–Bicaz Lake* (2010), recognized at the Euroinvent National Book Salon (2012).

As a case study, we evaluated water quality of the Izvorul Alb stream, a major right-bank tributary of the Bicaz Reservoir, at two monitoring stations (upstream S4 and downstream S5). We applied the IBGN methodology and adapted the Danish DSFI protocol to ensure representative sampling of benthic macroinvertebrates across multiple microhabitats. We calculated IBGN scores using random combinations of eight samples per station. Water quality was classified as good at both sites, with IBGN scores ranging from 14–16 at S4 and 15–16 at S5. This variation highlights that single sampling events, even when methodologically rigorous,

may not fully capture the ecological state, emphasizing the need for repeated sampling to obtain robust assessments.

Following the formation of the Bicz Reservoir, the affected ecosystems underwent a secondary ecological succession, with profound abiotic and biotic transformations. Rheophilic species, adapted to flowing waters, declined sharply, while stagnophilic communities expanded in the newly created lentic habitats. Key environmental parameters—temperature, oxygenation, light penetration, and hydrostatic pressure—shifted significantly, restructuring benthic communities and trophic networks. The long-term study revealed that river–lake succession progressed more rapidly and extensively in areas initially dominated by rheophilic species, underscoring the importance of initial ecosystem characteristics in shaping successional dynamics.

We observed that rheophilic macroinvertebrates, particularly aquatic larvae of *Plecoptera*, *Ephemeroptera*, *Trichoptera*, and *Diptera*, as well as *Gastropoda*, *Amphipoda*, and *Hydracarina*, were most affected due to their specialized morphological, physiological, and behavioral adaptations to flowing water. Only a fraction of these species survived the transition, while existing lentic ecosystems—ponds and side branches—served as nuclei for colonization by stagnophilic macroinvertebrates, plankton, macrophytes, and fish.

The initial nutrient enrichment following inundation triggered rapid growth of decomposer bacteria, phytoplankton, zooplankton, zoobenthos, and nekton. For instance, populations of *Alburnus alburnus*, a prolific plankton-feeding fish, exploded due to abundant zooplankton. Comparing pre- and post-successional benthic communities, we found an approximate 80% decline in rheophilic larvae, with surviving benthic fauna showing euribiont characteristics.

After five decades of succession, we evaluated the functional structure of the Bicz Reservoir ecosystem, examining phytoplankton, zooplankton, zoobenthos, and nekton, along with thermal regime, oxygenation, pH, conductivity, transparency, and nutrient concentrations. Integrating these results with pollutant assessments in permanent tributaries revealed a ~50% decline in lacustrine bioproductivity compared with the first two decades of succession. The trophic structure indicates a 10% ecological efficiency per link, with a reduction from 6000 tons of primary phytoplankton biomass to half after 50 years. This highlights how long-term secondary succession following river-to-lake transformation reshapes both biodiversity and ecosystem productivity.

1.1.8. Ecomorphological biomarkers

In this section, I explored the ecomorphological biomarkers of aquatic insect larvae used as bioindicators of running water quality, focusing on sensilla and osmoregulatory cells. I have shown that the pollution sensitivity of these larvae can be evaluated through detailed anatomical, histological, and cytological analyses, including the diversity, distribution, and density of specialized cells and tissues directly involved in organism–environment interactions.

Sensilla, located on the cuticle and in direct contact with the external medium, play a central role in perceiving environmental cues and maintaining homeostasis under hydrochemical stress. We investigated these structures using optical and electron microscopy (SEM and TEM), complemented by epifluorescence microscopy based on natural tissue autofluorescence. My studies demonstrated that predatory Plecoptera larvae (*Perla marginata*, *Dinocras cephalotes*, and *Perlodes microcephalus*) possess highly differentiated sensory arrays, forming a “sensory tissue” capable of detecting a wide range of physical, chemical, and biological stimuli. Sensilla exhibit diverse morphologies—including trichodea, chaetica, basiconica, coeloconica, and campaniformia—distributed on antennae and mouthparts, each adapted to mechanoreception, chemoreception, or thermo-hygroreception.

In parallel, we studied osmoregulatory adaptations through the structure and distribution of ionocytes in larval cuticles and gills. These specialized cells, including caviform, coniform, and floriform types, enable efficient ionic uptake in freshwater environments, sustaining hemolymph homeostasis despite low external ion concentrations. Overall, this work demonstrates that the combined ecomorphological and molecular study of sensilla and ionocytes provides a mechanistic understanding of the pollution sensitivity of aquatic macroinvertebrates. These organisms act as highly informative biosensors, displaying complex structural, cellular, and molecular adaptations that allow rapid integration of environmental stimuli, effective osmoregulation, and xenobiotic processing. My findings establish ecomorphological biomarkers as reliable tools for assessing ecological quality and form a foundation for linking structural, functional, and physiological adaptations in freshwater bioindicator species.

1.1.9. Conclusions

In the analysis of the water quality of the tributaries of the Bicaz Reservoir, assessed through aquatic macroinvertebrates, we found that the waters fall into the first-use category

(class I), reflecting good quality in accordance with the requirements of the Water Framework Directive 60/2000, which aimed for all surface waters to meet this standard by 2015.

Based on the trophic value of phytoplankton species, we classified the Bicz Reservoir as oligotrophic, and evaluations of zooplankton and benthic chironomid communities further confirmed good water quality with low organic load. Fish bioproduction was low due to the limited trophic resources of phytoplankton, zooplankton, and zoobenthos, influenced by large annual water level fluctuations of up to 40 meters. This reduced biological productivity reflects an oligo–mesotrophic status, demonstrating the relationship between water quality and piscicultural potential. In this context, applying the integrated aquaculture system (Ackefors & Rosen, 1979; Miron *et al.*, 1983) and optimizing salmonid growth techniques in the Bicz Reservoir enabled a commercial production of approximately 300 tons annually.

The ecomorphological studies on aquatic bioindicators, particularly mayfly and stonefly larvae, revealed essential structural and functional adaptations for maintaining osmotic balance in freshwater ecosystems. The presence, diversity, and variable distribution of ionocytes reflect the adaptation of these organisms to the specific chemical characteristics of their habitats. Microscopic analyses allowed us to characterize the detailed morphology and distribution of ionocytes and osmoregulatory epithelia, highlighting specialized structures such as floriform and caviform cells that actively regulate ions under salinity stress. These ecomorphological adaptations reinforce the role of these organisms as sensitive bioindicators of water quality changes.

Furthermore, this research demonstrates that ecomorphological investigations provide a strong foundation for molecular and omics-level studies, helping to explain the physiological mechanisms and differential responses of bioindicator species to pollution and other stressors. Integrating morphological and molecular approaches opens new perspectives for understanding the pollution sensitivity and resilience of aquatic species, offering valuable insights for monitoring and protecting freshwater ecosystems as part of natural capital.

1.2. Biomarkers with applications in Biomedical Sciences

1.2.1. Research motivation

In this subchapter, I present the need to advance cancer diagnosis and personalized treatment through the discovery and validation of novel molecular biomarkers. Over the past decade, significant progress has been made in oncology, yet conventional serum biomarkers, such as PSA, AFP, CA-125, HE4, and CEA, and histopathological analyses still face critical limitations. These biomarkers often lack sufficient specificity and sensitivity, and can be influenced by non-cancerous conditions, leading to false positives, overdiagnosis, and delayed or inappropriate treatment. Histopathology, while considered the clinical gold standard, can be invasive, time-consuming, and sometimes fails to capture the molecular and clonal heterogeneity of tumors. Early detection, accurate risk stratification, and dynamic monitoring of therapeutic responses remain challenging with traditional methods.

To address these challenges, we presented exploring innovative approaches, including minimally invasive or non-invasive molecular biomarkers, proteoform-resolved proteomics, imaging mass spectrometry (MALDI-MSI), and advanced computational techniques. We aim to integrate these tools to better reflect the complexity of tumor biology, providing more precise diagnostic, prognostic, and predictive information. This work emphasizes the importance of multidisciplinary collaboration between biologists, clinicians, chemists, engineers, and data scientists to translate research findings into clinical practice effectively. Ultimately, our goal is to contribute to the development of more reliable, rapid, and patient-centered strategies in oncology, aligned with the principles of predictive, preventive, personalized, and participatory medicine.

1.2.2. Role of Biology and Biologists in the identification of novel biomarkers

In this subchapter, I emphasize the essential role of biologists and biochemists in discovering and validating new biomarkers. Our expertise in experimental models, molecular mechanisms, and data interpretation bridges fundamental biological research with biomedical applications that have direct clinical impact. Biomedicine serves as a central link between laboratory discoveries and medical practice, accelerating the translation of biological knowledge into diagnostic, therapeutic, and predictive tools that shape modern medicine.

At the Faculty of Biology, we contribute to this progress through teaching advanced master's programs in Medical Laboratory and Molecular Genetics. Since 2021, I have taught

IHC, and from 2025 I have led courses on Molecular Biomarkers. These programs train specialists capable of integrating advanced analytical methods, emerging technologies, and interdisciplinary approaches needed for biomarker development and omics data interpretation. Designing and delivering these courses has required continuous learning and critical engagement with cutting-edge molecular biology and biomedicine, which has directly inspired and informed the research presented in this habilitation thesis.

Optical microscopy plays a central role in detecting and validating biomarkers, ranging from transmitted light and epifluorescence to confocal microscopy. When combined with IHC, IF, or *in situ* hybridization (RISH, FISH), these techniques allow precise spatial localization of biomolecules at the cellular and tissue levels. Importantly, microscopy complements proteomics by contextualizing molecular changes, enabling the integration of cellular imaging with molecular signatures. This synergy reflects the direction of modern biomedicine, aiming to decode complex pathogenic mechanisms and develop multiparametric biomarkers.

Overall, this research and teaching converge on using bioindicators and biomarkers as integrated tools to assess complex biological processes, both in ecological systems and biomedical science, providing a foundation for translational and innovative investigations.

1.2.3. Role of biomarkers in oncology

1.2.3.1. Biomarkers: definition, classification, and applications

A biomarker is any measurable biological feature—molecule, cell, tissue, physiological parameter, or imaging signal—that provides objective information about the biological or pathological state of an organism. An ideal cancer biomarker should be specific, sensitive, reproducible, cost-effective, and easy to use, enabling early disease detection, accurate reflection of tumor characteristics, and monitoring of therapeutic efficacy. Preferably, biomarkers should be obtainable via non-invasive or minimally invasive methods.

Biomarkers serve diagnostic, prognostic, predictive, monitoring, susceptibility, and safety purposes. Prognostic biomarkers estimate disease progression and identify high-risk patients, while predictive biomarkers guide personalized therapy by indicating the likelihood of treatment response. They can be molecular (DNA, RNA, proteins, lipids, metabolites), cellular (e.g., immune cell counts), or imaging-based (e.g., MRI-detected lesions). In oncology, biomarkers include genetic, epigenetic, proteomic, lipid, cell-free DNA (cfDNA/ctDNA), microRNA, metabolites, exosomes, serological, and imaging markers.

Biomarkers may originate from tumor clones, the tumor microenvironment, or the host response to tumorigenesis, and they can be detected in solid tissues (biopsies) or body fluids (liquid biopsies (LB), e.g., blood, urine, saliva). LB enable non- or minimally invasive detection of molecular and cellular biomarkers, including circulating protein fragments, allowing early diagnosis and personalized interventions. This approach supports continuous monitoring of disease dynamics and offers a promising complement or alternative to conventional tissue biopsies and imaging methods.

1.2.3.2. *In situ* identification and characterization of protein biomarkers

To overcome the limitations of classical histopathology in the multi-omics era, multicolor molecular microscopy and molecular imaging provide spatial and quantitative information on thousands of proteins and antigens, some of which serve as biomarkers, without requiring target-specific labeling. *In situ* analysis of protein biomarkers can be applied to human tissues, animal models, and cell cultures using histochemistry, IHC, IF, and MALDI-MSI.

MALDI-MSI offers distinct advantages over traditional histology, enabling the detection and mapping of structural proteins, bioactive molecules, enzymes, hormones, peptides, and other functionally relevant biomolecules directly in tissues. This technique is crucial for biomarker discovery and validation in neuroproteomics, oncology, aging research, parasitology, forensic medicine, and ecotoxicology. It also supports understanding physiological and pathological mechanisms such as tumor heterogeneity, wound healing, neuronal plasticity, apoptosis, oxidative stress, xenobiotic metabolism, and immune signaling.

MALDI-MS and MALDI-MS/MS provide sensitive, direct analysis of small and large molecules, from picomoles to femtomoles, in biological fluids, tissues, and cell lysates with minimal sample preparation. These methods enable precise structural characterization of proteins and peptides, including their PTMs and protein-protein interactions, offering complementary spatial and molecular data essential for biomarker discovery, functional studies, and personalized medicine applications.

1.2.3.3. Role of PTMs of protein biomarkers. Implications for immunohistochemical detection

PTMs affect 50–90% of human proteins and represent a major source of biological variability. PTMs, including phosphorylation, acetylation, methylation, ubiquitination, SUMOylation, neddylation, hydroxylation, and others, regulate protein stability, activity,

localization, and interactions. They significantly influence the detection of protein biomarkers by IHC as conformational changes or protein–protein interactions (PPIs) can mask or expose epitopes, affecting antibody binding and result interpretation.

Modern strategies, such as binding mode-guided antibody design, bioinformatic sequence analysis, and validation with positive/negative controls, are essential to ensure antibody specificity and reliable detection of PTM-modified proteins. Proteomic approaches complement genomics by enabling the identification of PTMs and PPIs, providing insights into tumor biology and malignant processes. PTMs are directly involved in all hallmarks of cancer, including proliferative signaling, evasion of growth suppressors, resistance to apoptosis, metabolic reprogramming, invasion, metastasis, angiogenesis, and immune evasion. In BC, PTMs of transcription factors such as HIF1 α and p53 modulate cellular responses to hypoxia, regulate gene expression, and contribute to tumor progression, metastasis, and therapy resistance. Understanding PTMs of these key regulators not only informs biomarker discovery but also guides the development of targeted and personalized therapeutic strategies, including interventions to mitigate tumor hypoxia and enhance treatment efficacy.

1.2.3.4. Multifunctional role of biomarkers in Biomedicine

Deep understanding of biomarkers is crucial for biologists and biochemists, providing both theoretical and practical tools to interpret biological and pathological mechanisms, identify and validate biomarkers, and analyze molecular and cellular data. Expertise in biomarkers is essential in medical and pathology labs, contributing to diagnostic, prognostic, and predictive test development, supporting personalized therapies, treatment monitoring, and early disease detection, including cancer.

Between 2019–2024, we collaborated with Clarkson University (Prof. dr. Costel Darie) publishing 24 papers and 1 book chapter, focusing on biomarker identification and characterization:

- **Multi-omics approaches:** Integrating genomics, transcriptomics, proteomics, metabolomics, interactomics, and epi-omics for BC progression, subtyping, and biomarker discovery.
- **Tandem mass spectrometry (MS/MS):** Analyzing proteins and peptides, identifying PTMs and protein interactions, providing essential data for diagnostic and predictive biomarkers.

- **New biomarkers in invasive ductal carcinoma (IDC):** Proteomic profiling identifies dysregulated proteins, enables early detection, prognosis assessment, and minimally invasive biomarker monitoring.
- **Exposomics:** Evaluates lifetime exposure to xenobiotics and environmental factors affecting BC initiation and progression; integrates diet, nutrition, and environmental impacts on molecular pathways.
- **Diet and nutrition effects:** Healthy, varied diets promote apoptosis, inhibit proliferation and metastasis, modulate stress responses, and enhance therapy efficacy. Contaminants in diet may have synergistic or antagonistic effects.
- **Biological disparities in BC:** Precision oncology requires understanding tumor molecular profiles across racial, ethnic, age, sex/gender, and environmental contexts; multi-omics helps explain incidence, mortality, and therapy response disparities.
- **Nanorobotics:** Nanorobots act as targeted drug carriers, modulate tumor microenvironments, induce apoptosis, reprogram metabolism, and monitor tumors in vitro and in vivo, with potential for personalized BC therapy.
- **Breast cancer-on-chip platforms:** BCoC, BCMoC, and LB-on-chip models replicate tumor cell continuum, intercellular interactions, invasion, EMT, metastasis, and drug testing; integrated with proteomics for biomarker discovery.
- **Microorganisms as anti-cancer agents:** Viruses, bacteria, fungi, protozoa, and microalgae can have oncogenic, oncolytic, or dual effects; used in therapy, gene delivery, photodynamic treatments, and inspiration for biomimetic nanorobotics and targeted therapies.

Overall, biomarkers provide critical insight into disease mechanisms, personalized medicine, therapy development, and early detection, while emerging technologies like nanorobotics, tumor-on-chip models, and microbial agents expand their translational potential.

1.2.4. Applications of Eco-Evo-Devo theory in the study of BC

Within the complex interplay of genetic, epigenetic, environmental, and evolutionary factors, identifying and characterizing specific BC biomarkers is essential for translating Eco-Evo-Devo concepts into clinical practice. Each eco-evolutionary and developmental process leaves a distinct molecular footprint in cells and tissues, and biomarker analysis can help decode these footprints, enabling more precise diagnostics and personalized therapy. From a biomedical perspective, BC behaves as a disease of the genome, epigenome, environment, and

developmental biology, with tumor expansion and metastasis explained through ecological, evolutionary, and developmental principles. Tumors can be conceptualized as “pseudo-organs” or dynamic ecosystems, consisting of cancerous and non-cancerous cells whose interactions, together with structural and metabolic constraints, shape resource availability, immune pressure, and proliferative success, driving tumor progression.

BC cells exhibit high complexity and diversity, hierarchical organization, self-renewal capacity, and phenotypic plasticity. They adapt metabolically, behaviorally, and physiologically to survive and thrive under environmental pressures. Tumor initiation and spread are viewed as short-term microevolutionary processes: cells acquire transformative, proliferative, and metastatic capabilities, shaped by selection in primary tumor sites or pre-metastatic niches. BC can also be seen as a co-evolving ecosystem of clones and cancer-associated cells, where competition, predation, mutualism, and parasitism influence tumor dynamics. Circulating tumor cells and disseminated tumor cells may colonize distant organs, remodeling local niches and forming metastases.

Cancer evolution mirrors ecological and evolutionary processes, with branched evolution analogous to speciation. Tumor cells often reactivate ancestral unicellular programs, enabling survival under hypoxia, acidosis, and stress, promoting dedifferentiation, invasion, migration, and colonization of new niches. This perspective supports the use of tumor microenvironment vulnerabilities to improve therapy, similar to how species extinction can occur in nature due to environmental pressures.

Finally, tumor regrowth after therapy resembles ecological succession: resistant clones survive, repopulate the tumor ecosystem, and colonize distant sites. The BC cancer continuum cascade concept (BCCCC) models the spatiotemporal evolution of heterogeneous tumor and associated cells from initiating mutations to primary tumors, circulating tumor populations, and secondary metastases. At the molecular level, this continuum is supported by dynamic proteomic profiles (BCPCC), which track phenotypic adaptations, epithelial-mesenchymal transition (EMT), intravasation, circulation, extravasation, and metastatic colonization, providing a roadmap for precise biomarker-based diagnostics and therapeutic strategies.

1.2.5. Role of Jumping Translocation Breakpoint (JTB) protein in breast cancer

Jumping translocations (JTs) are a type of aberrant genomic rearrangement that can fuse sequences from chromosome 1 with telomeric regions of “recipient” chromosomes, often generating partial trisomies of the 1q arm. These rearrangements are frequently observed in

multiple myeloma and various cancers, including BC. The Jumping Translocation Breakpoint (*JTB*) gene encodes a 146-amino-acid transmembrane protein (JTB) with a signal peptide, an extracellular region, and a cytoplasmic domain. JTs involving JTB can produce truncated proteins lacking the transmembrane domain, potentially disrupting its biological function and contributing to tumorigenesis. Amplification of 1q21, where JTB resides, is associated with oncogene dysregulation across multiple cancers.

From 2019 to 2024, our research focused on elucidating JTB's role in key cellular processes such as proliferation, apoptosis, oxidative stress, and mitochondrial homeostasis. Using the MCF7 BC cell line (luminal A subtype) and complementary HEK293 cells, We studied the effects of both overexpression and knockdown of JTB. Proteomic analyses, including in-solution proteomics and two-dimensional electrophoresis coupled with LC-MS/MS, revealed that altering JTB levels affects pathways involved in cell cycle regulation, oxidative stress, apoptosis, and energy metabolism, supporting its direct involvement in malignant transformation.

Bioinformatic tools—GSEA, STRING, GeneCodis, and Reactome—were applied to interpret the proteomic data. These analyses identified JTB as a regulatory node in protein networks associated with tumor behavior, highlighting its potential as a molecular biomarker and therapeutic target in BC. The results suggest that JTB may act as a tumor suppressor, influencing multiple signaling pathways critical for cancer progression.

Further investigations extended to in vivo studies in midgestation mouse embryos (E11.5–E13.5) using double-RISH, which allowed simultaneous detection of *Jtb* mRNA and neural crest markers. Expression was found to be widespread but heterogeneous, with particularly high levels in cardiac tissue, and showed co-expression patterns with neural crest markers *Foxd3* and *Sox10*, suggesting potential links between JTB and neuroectoderm-derived tumor origins. These findings indicate a complex epigenetic regulation of JTB expression, involving DNA demethylation, chromatin decondensation, and possible telomeric dysregulation.

Overall, our work demonstrates that JTB plays a multifaceted role in BC, influencing tumorigenic pathways and cellular behavior. Understanding its molecular function and regulation provides a foundation for further research into its diagnostic and therapeutic potential, both in vitro and in vivo, and highlights its relevance as a biomarker in BC.

1.2.6. Role of desmin (DES) and its PTMs in pancreatic cancer (PDAC)

I am a co-investigator in an NIH-funded grant proposal titled “*Identification and Characterization of Pathogenic Mutations of Desmin (DES) Responsible for Pancreatic Ductal Adenocarcinoma Development*”. The project focuses on understanding how DES mutations, particularly D399Y and its phosphorylated form, contribute to EMT, invasion, and metastasis in pancreatic ductal adenocarcinoma (PDAC).

Our laboratory provides comprehensive histological and microscopic support for this project. Equipped with automated tissue processors, paraffin embedding systems, semi-automatic microtomes, cryostats, automated staining stations, electron microscopy sample processors, critical-point dryers, sputter coaters, and a laser-scanning confocal microscope, we can process, section, stain, and image tissues with high precision and reproducibility. These facilities allow analysis of both fresh-frozen (FF) and formalin-fixed paraffin-embedded (FFPE) tissues, supporting routine and specialized histology, immunohistochemistry (IHC), and immunofluorescence (IF) studies.

The project has two main objectives. The first, led by the U.S. principal investigator, focuses on identifying DES mutations and PTMs in advanced PDAC, generating peptide-specific antibodies against DES D399, DES Y399, and phospho-DES pY399 for immunopurification and proteomic analysis of DES isoforms from tumor, adjacent normal tissue, and serum. The second objective, conducted by our team in Romania, aims to evaluate the diagnostic and prognostic relevance of DES D399Y/pY399 in advanced PDAC. Using FFPE patient tissues, we will perform IHC and co-localization studies with cell-type-specific markers (e.g., cytokeratin 19 for ductal cells, vimentin for mesenchymal cells) to quantify and map the expression of native, mutant, and phosphorylated DES isoforms. Preliminary hypotheses suggest that elevated expression of these DES isoforms correlates with EMT activation and disease progression in PDAC. Through this integrated experimental and imaging workflow, our research will help clarify the functional significance of DES mutations and PTMs in PDAC progression and their potential as biomarkers for diagnostic and therapeutic applications.

1.2.7. Applications of optical microscopy in the assessment of bacterial viability biomarkers

We have used differential interference contrast (DIC), FLUO, and confocal laser scanning microscopy (CLSM) to investigate the mechanisms of action of a new class of synthetic tricyclic flavonoids with potent antibacterial activity at low concentrations. These studies focused on evaluating bacterial viability through membrane integrity, which I employed as a biomarker for the cellular damage induced by these flavonoids.

Bacterial cells from *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli* were treated and stained with two fluorochromes, acridine orange and propidium iodide/ethidium bromide, to distinguish viable from damaged cells. Additionally, we applied FLUO and DIC microscopy to explore the synergistic enhancement of conventional antibiotics (oxacillin, erythromycin, gentamicin, ciprofloxacin, tetracycline, and clindamycin) by phenolic compounds extracted from mulberry (*Morus* spp.), namely morusin and kuwanon G. These combinations disrupted bacterial membrane integrity, confirming their potential as effective antimicrobial therapies. Through these optical microscopy approaches, we were able to link membrane damage to antibacterial efficacy, demonstrating the utility of membrane integrity as a reliable biomarker for assessing both novel compounds and combinatorial antimicrobial strategies.

1.2.8. Applications of optical microscopy for the identification of morphological biomarkers in Anthropology

We have applied DIC and fluorescence microscopy to study human hair, using it as a biomarker of post-mortem degradation in archaeological sites from the 18th–19th centuries. Microscopic analyses revealed that hair degradation is influenced both by intrinsic factors and by burial environment conditions. By examining transverse sections of hair shafts, we identified distinct stages of degradation: partial destruction of the cuticle with well-defined cortical tunnels indicated early degradation, while complete cuticle loss with interconnected microtunnels reflected advanced microstructural changes. We also analyzed biotic and abiotic factors affecting hair degradation, including fungal deposits, circumferential cracks, mechanical fractures, and cortical lesions revealed through autofluorescence. These studies confirmed that DIC and autofluorescence microscopy can effectively discriminate between natural and environmental degradation processes. Comparative analysis of human versus murine hair highlighted structural differences, supporting their use as differential species

biomarkers with potential forensic applications. Human hair typically showed cuticular damage, *Trichorexis nodosa*, and clustered medullary cells, whereas murine hair exhibited continuous columnar medullary cells, indicating a more resilient internal structure.

Furthermore, we applied DIC, polarization (POL), BF, and autofluorescence to visualize cuticle, cortex, melanosomes, and degradation microtunnels in fine detail. This integrated approach allows the identification and quantification of structural biomarkers, enhancing tafonomic and comparative analyses. Additionally, I contributed to histological analyses of pathological bone fragments, such as humeral hyperostosis potentially linked to treponematosi, which enabled detailed characterization of bone remodeling and proliferation associated with pathology, providing valuable data for paleopathology and anatomical archaeology. These studies were part of the PhD research of Asist. univ. Ozana-Maria Ciorpac-Petru, under the supervision of Prof. univ. habil. dr. Luminița Bejenaru. I was a member of the doctoral advisory committee and mentored the candidate, contributing to the design, interpretation, and dissemination of the results.

1.2.9. Role of optical microscopy in the analysis of urinary sediment as biomarker

Urinary sediment analysis is a valuable diagnostic tool that provides information on cells and crystals, which serve as morphological biomarkers for various clinical conditions. The unique optical properties of crystals, including birefringence and interference patterns, allow precise identification and differentiation, facilitating early detection of kidney stones and metabolic disorders. By combining optical microscopy with morphological and biochemical interpretation, this approach offers a rapid, non-invasive, and reliable method for monitoring urinary system health and preventing complications.

DIC and POL microscopy are particularly effective for examining urinary sediments, offering advantages over conventional BF microscopy. These techniques enable the detection and characterization of urinary cells and crystals such as calcium oxalate, uric acid, and struvite, which indicate metabolic abnormalities, urinary tract disorders, or biomineralization-driven stone formation. The birefringent and interference properties of crystals under POL allow accurate differentiation of crystal types, which can be challenging with traditional transmitted-light microscopy. For example, calcium oxalate crystals appear refractile and brightly shaped, while uric acid crystals show pleomorphic yellow-brown birefringence.

This approach is especially valuable for detecting crystals at early stages or in low concentrations, supporting timely therapeutic or preventive interventions. POL also enhances

diagnostic accuracy by reducing misidentification caused by amorphous salts or cellular debris, establishing its role as a standard tool in nephrology and clinical pathology laboratories. Urinary sediment analysis using DIC and POL provides detailed visualization of epithelial cells, crystal types, and microstructural features, strengthening its application as a biomarker-based diagnostic method.

SECTION II. DEVELOPMENT OF TEACHING AND SCIENTIFIC CAREER

2.1. Past and Present

Since 2024, I have been an Associate Professor (Conferențiar univ. dr.) in the Department of Biology at the Faculty of Biology, “Alexandru Ioan Cuza” University of Iași, after progressing through all previous teaching and research positions here (research assistant, teaching assistant, university assistant, and senior lecturer/CSIII). I have been part of the Faculty of Biology team since 1997, accumulating nearly 30 years of uninterrupted experience in teaching and research.

The main postdoctoral achievements of my career are highlighted below:

- My academic journey began with the award of a PhD in Biology, following the public defense of my dissertation, “*Eco-morphological Research on Certain Plecoptera and Ephemeroptera Larvae from the Tributaries of Lake Bicaz*”.
- I consider the modernization of the **Laboratory of Animal histology and Confocal microscopy** to be the most significant achievement of my career. This laboratory allows all students, regardless of specialization, to learn how to prepare permanent microscopic slides. This initiative has facilitated graduates’ integration into medical and research laboratories, as well as schools across Romania and abroad, earning recognition for their practical skills and theoretical knowledge. All laboratory equipment was acquired through research projects I coordinated as principal investigator.
- I have been director of three research projects (Assessment of polluosensitivity of some bioindicators of water quality using morpho-histological characteristics; Extension of the laboratory of Aquatic ecology Potoci for assessing the mountain reservoirs pollution induced by floating trout farms, according to the Water Framework Directive 2000/60/CE; Development of the infrastructure of the laboratory of bioindicators of water quality ACVALUR Potoci to assess the polluosensitivity of aquatic organisms)

with a total budget of 533.500 €. I also participated as a member or external collaborator in nine other national research, institutional development, and educational projects.

The opportunity to lead these projects has been pivotal to my career, allowing me to produce original and relevant scientific results in biology, coordinate complex research teams of specialists, PhD students, master's students, undergraduates, and support staff, generate exploratory research directions, and manage activities to meet project objectives while fostering learning and research.

- My teaching activity has been extensive and diverse, consistently integrating scientific research results into the programs and specializations of the Faculty of Biology at UAIC Iași. Courses and competencies developed in bioindicators, biomarkers, histology, developmental biology, and applied ecology are reflected in numerous subjects taught. These courses provide a strong theoretical and practical foundation, preparing students and master's candidates for modern research and advanced laboratory techniques.
- I also oversaw the construction and equipment of the **ACVAPUR** at the Potoci Biological Station, a complex, high-impact project that facilitated numerous PhD dissertations (supervisor Prof. dr. Ionel Miron) and enabled large-scale international collaborations with the University of Konstanz (Germany), Uppsala University (Sweden), and other partner universities.
- I contributed to equipping the Faculty of Biology's **Laboratory of Electron microscopy**, acquiring and implementing instruments for preparing SEM and TEM samples. These resources support both teaching and research, contributing to undergraduate, master's, and PhD studies, and are used as demonstrative tools in optional Microscopy courses.
- The Laboratory of Animal Histology and Confocal Microscopy has maintained nearly a decade of **collaboration** with the Biochemistry and Proteomics Laboratories at Clarkson University (USA). This collaboration has led to multiple original and review articles, a book chapter published by Springer, and further publications in preparation. I am grateful to Prof. Costel Darie for opening the field of molecular histology and proteomics to me, which has been vital for research development and collaborative competitive grants.
- I have helped develop significant international partnerships, including participation in three European projects for implementing the European Master's program "*Ecological Management of Catchments in Europe*" (**ECOCATCH**) at Uppsala University

(Sweden), in collaboration with universities in Sweden, the UK, the Netherlands, France, and Portugal. I participated in 12 international workshops under the ECOCATCH program and co-organized the UAIC and Potoci Biological Station meeting in 2007.

- My publications include 12 books and book chapters with national and international publishers, 24 ISI-indexed articles as first author, 15 ISI-indexed articles as contributor, 22 BDI-indexed articles, and four popular science articles.
- My work has been recognized with prestigious awards from the Romanian Academy, including the “Grigore Antipa” and “Daniel Danielopolu” prizes, for contributions in both environmental and biomedical research.
- I completed two Erasmus teaching stages at the University of Vigo (Spain) and Uppsala University (Sweden).
- I completed the laboratory manager certification course “*Requirements for the Competence of Testing and Calibration Laboratories – SR EN ISO / IEC 17025:2005*”, organized by RENAR Bucharest.
- I have promoted the Faculty of Biology’s activities through presentations at schools and universities in Iași, across Romania, and internationally, and by participating in events such as *Open Doors at UAIC*, *Serata Procopiu*, *Researchers’ Night/Science Night*, *Biology in the Park*, national competitions, and annual student preparation sessions for the National Biology Olympiad.
- I have served on the supervision committees of four PhD students (three in Biology and one in Medicine, Pharmacology specialization) and have coordinated 75 undergraduate and master’s theses.
- A central focus of my academic activity has been student engagement, fostering their theoretical and practical development through adaptive teaching methods, integration of research into education, and promotion of critical thinking. I actively encourage student, master’s, and PhD participation in teaching, research, national and international scientific events, and publications, cultivating a culture of scientific responsibility and excellence. The results of my mentorship have been published in collaboration with students and doctoral candidates, including studies on cancer pathways, protein biomarkers, and comparative anatomical studies.
- Overall, my nearly three-decade-long teaching and research career has focused on:
 - 1. modernization of research and educational infrastructure,**

2. **integration of scientific research into education,**
3. **development of national and international collaborations.**

Through research project coordination and modernization of laboratories in histology, confocal microscopy, electron microscopy, and water quality evaluation, I have contributed to creating training and research environments that directly enhance the preparation of undergraduate, master's, and PhD students.

2.2. Future perspectives

The habilitation thesis outlines a vision for the future development of my academic career, emphasizing integrative research and teaching priorities. I aim to revitalize the fields of Ecology and Environmental Protection through modern approaches that combine bioindicators and molecular biomarkers to assess and monitor environmental quality. By integrating analyses at the tissue, cellular, and molecular levels, the **Molecular Ecology** seeks to deepen understanding of the impact of environmental factors on organisms. Courses such as “The Xenobiotic Cycle in Nature” will continue to develop students’ abilities to study absorption, distribution, biotransformation, elimination, bioaccumulation, toxicity, and biomagnification of chemical compounds, fostering the training of specialists capable of addressing the environmental challenges posed by pollution and industrial expansion.

In parallel, I intend to strengthen the interface between Biology and Medicine through the field of **Biomedicine**, establishing an integrative research and teaching profile that combines fundamental knowledge with clinical applications. This approach focuses on identifying and validating molecular biomarkers using IHC, tissue structural analysis, and proteomics, offering insights into both basic biological mechanisms and translational applications in diagnostics, prevention, and personalized medicine. It provides students with comprehensive theoretical and practical training, while involving them in original research projects, theses, and doctoral studies, thus preparing a new generation of competitive scientists.

A central teaching objective is the publication of **high-quality university textbooks and manuals** for undergraduate and master's courses, ensuring students have access to current scientific discoveries alongside practical laboratory skills. Simultaneously, I aim to **develop research projects with national and international funding**, fostering collaboration with specialists, young researchers, and students to produce original, widely recognized results disseminated through publications, conferences, and academic networks. Acquiring modern equipment and digital tools for Laboratory of animal histology and confocal microscopy will

enable advanced biomarker studies, particularly in oncology field and ecology, and support effective student training.

Future collaborations will extend ongoing partnerships with institutions such as Clarkson University, the University of Medicine and Pharmacy “Grigore T. Popa” in Iași, and the Regional Institute of Oncology, while also establishing new interdisciplinary links with experts in bioinformatics, biophysics, applied mathematics, and artificial intelligence (AI). These collaborations will support the analysis of complex biological datasets and the development of innovative approaches at the interface of ecology, molecular biology, and biomedical research.

Equally important is the continued **investment in human capital** through mentoring and training students, master’s and doctoral candidates, and young researchers. Initiatives such as a **Summer School in Histology and Molecular Biomarkers** will provide intensive interdisciplinary instruction, hands-on experience in imaging and proteomic techniques, and opportunities to engage with international experts. These activities aim to cultivate independent, critical thinkers capable of addressing complex biological and biomedical challenges while integrating into the international scientific community.

Finally, **public dissemination of scientific knowledge** will remain a key focus. Interactive lectures, educational materials, traveling photomicrograph exhibitions, and outreach initiatives aim to engage pre-university and university audiences, stimulate interest in STEM disciplines, and enhance scientific literacy. These efforts also strengthen the visibility of the Faculty of Biology, attract new students to its programs, and promote a dynamic, modern image of biological and biomedical research. Through this integrative approach, I seek to combine research excellence, advanced teaching, and societal engagement, creating a sustainable framework for innovation, education, and knowledge transfer.