BIOGRAPHICAL SKETCH

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DO NOT EXCEED FIVE PAGES.

NAME: Bojana Gligorijevic

eRA COMMONS USER NAME (credential, e.g., agency login): bgligori

POSITION TITLE: Associate Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Belgrade University, Belgrade, Serbia	BS	2001	Chemistry
Georgetown University, Washington, D.C.	MS	2002-2005	Chemistry/ Biophysics
Georgetown University, Washington, D.C.	PhD	2005-2007	Biophotonics
Albert Einstein College of Medicine, Bronx, NY	Postdoc	2007-2012	Tumor Microenvironment
Albert Einstein College of Medicine, Bronx, NY	Instructor	2012-2015	Systems & Computational Biology

A. Personal Statement

My lab investigates the dynamics of cancer cell metastasis, with a focus on invadopodia, ECM-degrading protrusions on the cancer cell membrane. To address intrinsic and extrinsic, microenvironment-driven mechanisms of metastasis, my lab uses established and develops new intravital microscopy technologies. We integrate microscopy, image processing & machine learning with cancer biology, microfabrication and mathematical models. In particular, by developing the first longitudinal imaging window in mice, and combining it with photoconvertible fluorescent proteins, we were able to demonstrate itineraries of individual cancer cells from the primary tumor, through the blood vessels and inside the lung (*Nature Methods, Nature Protocols*). Next, by using intravital multiphoton microscopy and support vector machine classification, we were the first to demonstrate invadopodia assembly is a necessary step for blood vessel entry (*Plos Bio*). Most recently, using time-lapse of FUCCI-labeled tumors, spheroids and cells in confinement, we demonstrated that during G1 phase of the cell cycle, the cancer cells move at high velocities (*APL Bioengineering*) and assemble invadopodia, which is controlled via cytoplasmic pool of p27 and its interaction with Tks5 and cortactin in invadopodia (*J Cell Science*).

My laboratory offers the opportunity to train in developing and implementation of cancer imaging techniques and cancer mechanobiology. Mentees are exposed to a diverse set of microscopy-centric approaches applied to cancer, both *in vitro* and *in vivo*. My teaching and service at Temple are devoted to implementing microscopy towards solving biomedical questions. As a Director of Light Microscopy shared resources at Temple Engineering, me and my mentees have trained >100 students and collaborated with >20 labs on their microscopy applications. In addition, I have developed a hands-on course on Biophotonics for students of Bioengineering. This course has evolved as a result of my long experience of teaching microscopy, which includes MBL course in Woods Hole, as well as teaching microscopy to PhD and medical students at Albert Einstein College of Medicine. On international level, I have so far contributed a number of chapters in microscopy books; I am an active member of BINA Imaging Network and currently on organizing committee of Intravital Imaging of Cancer seminars, together with peers from France and Australia. I am excited to extend my collaborations, training and mentoring to FCCC as a Co-Director of BIF and have now established a satellite office and lab there for this purpose.

1. B. Gligorijevic*, D. Kedrin*, J. Wyckoff, V.V.Verkhusha, J. Condeelis, J. E. Segall, J. van Rheenen,"Intravital imaging of metastatic cell behavior through an orthotopic Mammary Imaging Window" **Nature Methods** (2008), 5:1019-1021 PMCID: PMC2820719
2. B. Gligorijevic, A. Bergman, J. Condeelis: "Multiparametric Classification Links Tumor Microenvironments with Tumor Cell Phenotype", **Plos Biology** (2014) 12: 1-15, e1001995. PMCID: PMC4227649

3. B. Bayarmagnai, L. Perrin, K. Esmaeili, X. Graña, E. Tüzel, B. Gligorijevic. "Invadopodia-mediated degradation is enriched in the G1 phase of the cell cycle", **Journal of Cell Science** (2019) doi: 10.1242/jcs.227116.
4. L. Perrin, T. Tucker, B. Gligorijevic, "Time-Resolved Fluorescence Imaging and Analysis of Cancer Cell Invasion in the 3D Spheroid Model", Preclinical models and imaging modalities of tumor microenvironment in metastasis, **Jove** (2021), doi 10.3791/61902

B. Positions and Honors

Positions

1998-2000	Undergraduate research assistant, University of Belgrade, Department of Chemistry, advisor: Professor Slobodan Milosavljevic
1999	Research Assistant, Federal Bureau for Measurements and Precious Metals, Laboratory for Ionizing Radiation (Belgrade, Serbia)
2001-2002	Research Talent, University of Belgrade, Faculty of Technology, Department of Environmental Engineering, Laboratory of Professor Dusan Antonovic
2000-2002	Research Talent, Military Medical Academy, Poison Control Center, Laboratory of Toxicology Research
2002-2007	PhD student, Georgetown University, Department of Chemistry, advisor: Prof. Paul D. Roepe
2007-2012	Post-doctoral fellow, Albert Einstein College of Medicine, Department of Anatomy & Structural Biology, Program in Microenvironment and Metastasis, Gruss-Lipper Biophotonic Center. Advisor: Professor and Chairman John Condeelis
2012-2015	Instructor, Albert Einstein College of Medicine, Systems and Computational Biology Department, Supervisor: Professor and Founding Chairman Aviv Bergman
03/01/2015-07/01/2020	Assistant Professor, Bioengineering Department, Temple University
09/01/2015-current	Primary Member, Cancer Signaling Program, Fox Chase Cancer Center
05/28/2020-current	Director of Light Microscopy, Bioengineering Department, Temple University
07/01/2020-current	Associate Professor, Bioengineering Department, Temple University
01/01/2021-current	Co-Director of Biological Imaging Light Facility, Fox Chase Cancer Center

Honors

1990	Exceptional Student Award "Vuk Karadzic", Sava Kovacevic School
1992	Young Researcher Award "Nikola Tesla", Belgrade City Council
2001-2002	Research Fellowship, University of Belgrade
2000-2002	Research Talent Fellowship, Military Medical Academy (Belgrade, Serbia)
2002	Exceptional Graduate Student, Espenscheid Fellowship (top 2%), Georgetown University
2003	Research Travel Grant, Georgetown University Graduate School of Arts and Sciences
2008	Belfer Poster Prize for Outstanding Postdoctoral Research (5th Annual, top 3%)
2008	American Society of Cell Biology conference, San Francisco, abstract chosen as 1 in 12 out of 1,229 abstracts as "New and Newsworthy"
2008	Listed in Nature Milestones: Light Microscopy collection, In situ and in vivo microscopy section
2010	Dennis Shields Award Postdoctoral Research Prize (three annual recipients, 5K for personal use)
2014	Career Transition Award K22 NIH- (denied due to K99 award acceptance)
2014	ATIP Avenir European Young Group Leader interview- denied for Temple Bioengineering position
2015,16,17,18,19,20	Temple University Faculty Merit Awards for Exceptional Research and Service
04/2021	Annual Fellow Invited seminar, Pediatric Oncology Branch, NCI

Professional affiliations

Journals

- Editorial Board Member, Frontiers in Cell & Developmental Biology
- Guest Editor, Invadosomes, European Journal of Cell Biology

Conferences/ workshops	o 2019, 2020, 2021-Scientific Co-chair, ASCB-EMBO Cell Bio Virtual Annual Meetings Special Interest Subgroup: Cell-Matrix and Cell-Cell Interactions in 3D Environments o European Society for Molecular Imaging (ESMI) meeting, reviewer/judge o Organizing Vice Chair, Integrative Mechano-Chemical Signaling in Invasion, Nice, France o Tumor Microenvironment Network NCI- Junior Investigator Committee o Program Chair and Co-organizer, Philly Motility Biannual Symposium o Faculty, Woods
Ad Hoc Reviews	• NIH, NCI Special Emphasis Panel ZCA1 SRB-X (O1) R, U54 Cellular Cancer Biology Imaging Research Centers (CCBIR) • NIH, NIDDK, Diabetes, Endocrinology and Metabolic Diseases-B • NIH, National Cancer Institute, Tumor Progression and metastasis section • Department of Defense, Pathobiology sections, Breast Cancer Research Program • National Science Foundation, Systems and Molecular Biology, GRF program • American Cancer Society, Cancer Structure and Metastasis Study section • AACR INNOVATOR Award in Tumor Microenvironment, Landon Foundation • National Science Foundation, ARI- R^2, Chemistry study section
Expert Grant Reviews	o PA Breast Cancer Coalition (PABCC) o Wellcome Trust, Dept Biotechnoloy, India Alliance o Breast Cancer Now foundation, United Kingdom o The Science Fund of the Republic of Serbia o Estonian Research Council (ETIS) o National Science Foundation of Poland o Swiss National Science Foundation

Ad hoc reviews: Nature Nanotechnology, Nature Protocols, Nature Scientific Reports, Science Advances, Cancer Research, Cancer Reports, Journal of Cell Biology, European Journal of Cell Biology, Integrative Biology, Disease Models & Mechanisms, Plos Biology, Plos One, Bioinformatics, APL Bioengineering, Frontiers in Cell and Developmental Biology, Acta Biomaterialia, Cell Adhesion and Migration, Experimental Cell Research, Journal of Visual Experiments, Methods, Molecules, Oncogene, Cells, Biophysical Journal.

Professional Memberships: American Society for Cell Biology, Biophysical Society, Invadosome Consortium, SigmaXi society, Metastasis Research Society, American Society for Matrix Biology

C. Contribution to Science

I. The genetic basis of resistance in malaria is very similar to stomach cancers, involving genes such as (pf)mdr1 and (pf)crt. Due to the lack of suitable technology, several opposing theories on underlying mechanisms of resistance were never tested. Led by this need, I have initiated my first independent collaboration with physicists as a PhD student. Together with a physics postdoc, I customized a Spinning Disk Microscope and developed several malaria live imaging technologies. This allowed me to test the malarial stage-specificity of drug chloroquine and show that its mechanism of action is based on the inhibition of heme dimerization. My two first-authored manuscripts in Biochemistry were covered in Editor letter in Biophotonic International. These results started a new branch in my advisor's lab. Also, the SYBR Green assay we have developed as a team is highly cited and used as the official malarial drug test at Walter Reed Institute.

a. B. Gligorijevic, T. N. Bennett, R. McAllister, J. S. Urbach and P. D. Roepe, "Spinning Disk Confocal Microscopy of Live, Intraerythrocytic Malarial Parasites. 2. Altered Vacuolar Volume Regulation in Drug

Resistant Malaria", **Biochemistry** (2006), 45(42):12411 – 12423. ("Hot Article of Biochemistry", 11/2006) PMCID: PMC400551

b. B. Gligorijevic, R. McAllister, J. Urbach and P. D. Roepe, "Spinning Disc Confocal Microscopy of malaria 1.Quantification of Hemozoin Development for Drug Sensitive versus Resistant Malaria", **Biochemistry** (2006), 45(42):12400 - 12410 PMID: 17029396
c. B. Gligorijevic, K. Purdy, D. A. Elliott, R. A. Cooper and P. D. Roepe "Stage independent chloroquine resistance and chloroquine toxicity revealed via Spinning Disc Confocal Microscopy" **Molecular Biochemical Parasitology** (2008), 159(1):7-23 PMCID: PMC2440633
d. T. N. Bennett, M. Paguio, B. Gligorijevic, C. Sidieu, A. D. Kosar, E. Davidson, P. D. Roepe "Novel, Rapid and Inexpensive Cell- Based Quantitation of Antimalarial Drug Efficacy" **Antimicrobial Agents and Chemotherapy** (2004), 48(5):1807-1810.

II. I advanced intravital multiphoton microscopy to study perivascular niche in tumor models, in living animals. I developed methodologies to simultaneously visualize several components of tumor microenvironment through time, at subcellular resolution; to be able to manipulate cell motility in primary tumor and finally, to monitor cell motility *in vivo* over days. This task demanded the formation of new collaborations with chemists developing fluorescent proteins, engineers constructing microfluidics and immunologists studying host-cell behavior in tumor progression. Among others, it resulted in high profile study in *Nature Methods* (cited >200 times/7 years), also featured in *Nature Milestones in Light Microscopy* collection.

a. B. Gligorijevic*, D. Kedrin*, J. Wyckoff, V.V.Verkhusha, J. Condeelis, J. E. Segall, J. van Rheenen, "Intravital imaging of metastatic cell behavior through an orthotopic Mammary Imaging Window" **Nature Methods** (2008), 5:1019-1021 PMCID: PMC2820719
b. B. Gligorijevic*, W. K. Raja*, J. Wyckoff, J. Condeelis, J.Castracane, "A new chemotaxis device for cell migration studies", **Integrative Biology** (2010), 2:696-706 PMCID: PMC4026270
c. D. Entenberg, J. Wyckoff, B. Gligorijevic, E. T. Roussos, V.V.Verkhusha, J.Pollard, J. Condeelis "Setup and use of a two-laser multiphoton microscope for multichannel intravital fluorescence imaging", **Nature Protocols** (2011),6:1500–1520, PMCID: PMC4028841
d. L. Perrin, B. Bayarmagnai, B. Gligorijevic, "Frontiers in intravital multiphoton microscopy of cancer", **Cancer Reports** (2019), e1192. https://doi.org/10.1002/cnr2.1192

III. Using my unique approaches, I was able to demonstrate that ECM-remodeling protrusions in cancer cells ("invadopodia") are necessary for intravasation and metastasis *in vivo*, and that they can be inhibited via microenvironment modulation. This work solved a 30-year-long controversy on invadopodia relevance for metastasis and microenvironmental cues which lead to assembly. Prior literature has mainly investigated invadopodia formation on cancer cells plated on dishes coated with extracellular matrix proteins. Under controlled conditions of the 2D culture, cells spread and invadopodia appear as small (1 µm) punctate enrichments of actin surrounding nucleus and create holes in the matrix. In the heterogeneous, dynamic, 3D tissue conditions it was necessary to establish new tools for identification and analysis of invadopodia (topographical, morphological, structural and functional). This work suggested that invadopodia may be a good predictor of metastasis and clinical target.

a. B. Gligorijevic*, J. Wyckoff*, H. Yamaguchi, Y. Wang, J. Condeelis: "N-WASP-mediated invadopodium formation is involved in intravasation and lung metastasis of mammary tumors", **Journal of Cell Science** (2012), 125:724-734 PMCID: PMC3367832
b. E. T. Roussos, M. Balsamo, S. K. Alford, J. B. Wyckoff, B. Gligorijevic, Y. Wang, M. Pozzuto, R.Stobezki, S. Goswami, D. A. Lauffenburger, A.R. Bresnick, F. B. Gertler and J. S. Condeelis, "Mena invasive promotes multicellular streaming motility and transendothelial migration in a mouse model of breast cancer", **Journal of Cell Science** (2011), 124:2120-2132 PMCID: PMC3113666
c. E. Genot, B. Gligorijevic, "Invadosomes in their natural habitat", **European Journal of Cell Biology** (2014), PMCID: PMC4262535
d. F. Tonisen, L. Perrin. K, van der Dries, A. Cambi, B. Gligorijevic, "EP4 receptor role in invasion of breast carcinoma" **European Journal of Cell Biology** (2017) PMCID: PMC5362301

IV. In summary, my work on intravital imaging tools transformed the cancer biology field in two distinct manners: First, by developing the first method for repeated, quantitative imaging of the same cell populations in living animals, we allowed researchers to view metastasis directly and study individual steps. The project resulted in a *Nature Methods* publication cited almost 300 times since 2009 (Google Scholar), followed by several studies

which used this technology to tackle molecular mechanisms in metastasis, each with > 100 citations. Second, I demonstrated that ECM-remodeling protrusions in cancer cells ("*invadopodia*") are necessary for cancer cell intravasation and metastasis *in vivo*, and that they can be inhibited via microenvironment modulation. During my K99 period, I worked on integration of intravital imaging with systems view. I have proposed the workflow to follow (see a. and b.) towards statistical classification of cell motility phenotypes in cancer model as a context-based decision. My experimental work has soon demonstrated this approach as predictive (see a.) and influenced both mathematical and cancer biologists' view on cell plasticity.

a. B. Gligorijevic, A. Bergman, J. Condeelis: "Multiparametric Classification Links Tumor Microenvironments with Tumor Cell Phenotype", **Plos Biology** (2014) 12: 1-15, e1001995. PMCID: PMC4227649
b. Bergman, J. Condeelis, B. Gligorijevic, "Invadopodia in Context", **Cell Adhesion and Migration** (2014) 8:273-279. PMCID: 4198352
c. Bergman, B. Gligorijevic: "Niche Construction Game Cancer Cells Play", in Physics of Cancer collection, **European Physics Journal Plus** (2015) 130:203 PMCID: PMC5027994
d. B. Bayarmagnai, L. Perrin., K. Esmaeili, B. Gligorijevic: "Intravital imaging of cell migration", **Methods in molecular biology** (2018), 1749:175-193. PMCID: PMC5996994.

V. My lab is currently devoted to paving a path to integration of biophotonics and systems view towards revealing mechanisms of cell decision-making in cancer and metastasis. With recent technological advances, we have the opportunity to combine the 4D in vivo/in vitro microscopy and computational/mathematical models into Systems Microscopy. Integrative approach to studying the tissue microenvironment and niches within incorporates and extend reductionist findings, giving rise to hypotheses at a system level.

a. K. Esmaeili, A. Bergman, B. Gligorijevic "Extracellular matrix cross-linking regulates invadopodia dynamics." **Biophysical Journal** (2018), 114:1455-1466. PMCID: PMC5883616
b. K. Esmaeili, E. Cardenas De La Hoz, AR. Cohen, B. Gligorijevic "Contact guidance is cell cycle dependent" **APL Bioengineering** (2018), 2(3):031904. PMCID: PMC5997297.
c. Bayarmagnai B., Perrin L., Esmaeili K., Graña X., Tüzel E., Gligorijevic B. **"**Invadopodia-mediated degradation is enriched in the G1 phase of the cell cycle", **Journal of Cell Science** (2019) doi: 10.1242/jcs.227116.
d. L. Perrin, B. Bayarmagnai, E. Tüzel and B. Gligorijevic, "Invadopodia enable cooperative invasion and metastasis of breast cancer cells", **BioRxiv** (2021) doi: https://doi.org/10.1101/2021.02.13.431047

My Bibliography: https://www.ncbi.nlm.nih.gov/pubmed/?term=gligorijevic+bojana

D. Research Support

Ongoing Research Support

NIH R01 CA230777 Gligorijevic (PI) 04/18/2019-03/31/2024
Targeting invadopodia-related mechanisms of cancer cell invasion and metastasis
Role: PI

ACS Research Scholar Grant Gligorijevic (PI) 09/01/2020-08/31/2024
Coordination of Cell Cycle and Invasion in Tumor Microenvironment Context
Role: PI

Temple BioEngineering Startup Funds, Gligorijevic (PI) 03/01/2015-open
Establishing Cancer Microscopy & Mechanobiology lab
Role: PI

Completed Research Support (last 3 years)

K99/R00 5CA172360 Gligorijevic (PI) 08/07/2013-07/31/2019
Systems microscopy of tumor cell motility in microenvironment context.
Role: PI

Conquer Cancer Now, Concern Foundation, Gligorijevic (PI) 07/01/2016-06/31/2018
Real-Time Intravital Imaging of Cancer Cell Cycle and Motility States
Role: PI