Research Highlight #301

Alexander Cartagena-Rivera, Ph.D.

National Institute of Biomedical Imaging and Bioengineering

Novel Use of Atomic Force Microscopy for Investigating Tissues

Atomic force microscopy for the life sciences has a wide range of uses, including quantitative live-cell mechanical property mapping or fast scanning of dynamic biological processes. Alexander Cartagena-Rivera, Ph.D. is the Chief of the National Institute of Biomedical Imaging and Bioengineering (NIBIB) Section on Mechanobiology and, with many resources at his research lab's disposal, is in a unique position to gain a profound level of expertise in tissue investigation using atomic force microscopes (AFMs). After his academic journey spanning Mayagüez, Puerto Rico, West Lafayette, Indiana, and now Bethesda, Maryland, Dr. Cartagena-Rivera currently has a team of six scientists at the NIBIB using advanced atomic force microscopy techniques to understand a variety of biological systems in the lab and in the clinic.

"My lab is divided into two main divisions. One is for the development of advanced atomic force microscopy methods that can be applied to understand complex living systems, depending on if it's in an organ, animal, some isolated tissue, in vitro cells, or in vitro molecules. Depending on the sample, we will develop advanced methods applicable to those lengths and timescales. The second portion is now the implications of our understanding of cellular and tissue mechanobiology for disease and therapeutics. So basically, how can we apply this advanced AFM-based method that we develop to understand disease progression? If it's cancer, if it's hearing, or if it's inflammation. Those are the three main biological questions that fall under the broad umbrella that we try to answer, and how are therapeutics used or could be used."

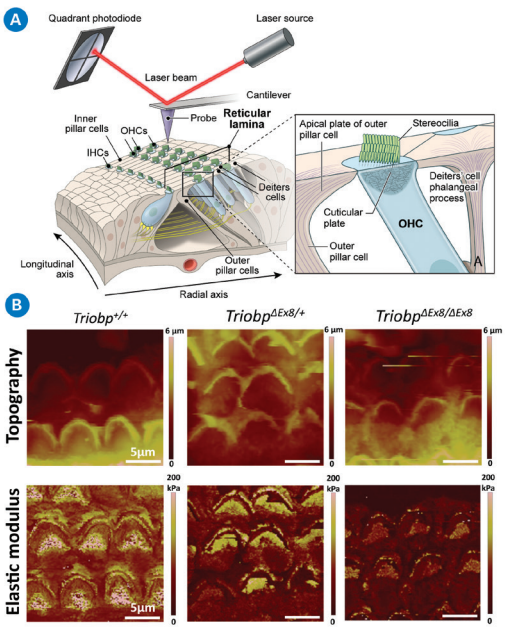
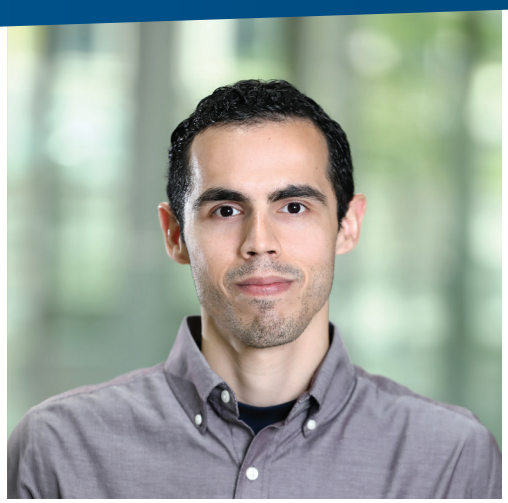


FIGURE 1.
Living mammalian organ of Corti apical surface nanomechanical mapping by AFM; (A) Schematic of the organ of Corti and the experimental AFM method; (B) Topography and Young's modulus maps for wild-type, Triobp-4/5 heterozygous, and Triobp-4/5 homozygous mice. (Adapted with permission from Babahosseini et al.[1])



ABOUT THE RESEARCHER

Alexander Cartagena-Rivera, Ph.D. is the Chief of the National Institute of Biomedical Imaging and Bioengineering (NIBIB) Section on Mechanobiology. He obtained his bachelor's degree in mechanical engineering from the University of Puerto Rico at Mayagüez in 2010 and received his Ph.D. in Mechanical Engineering from Purdue University in 2014. He then started his post-doctoral IRTA Fellowship position at the National Institute on Deafness and Communications Disorders in 2014 before taking a Tenure-Track Earl Stadtman Investigator position with the NIBIB in 2019. He is also currently a member of the NIH Distinguished Scholars program for underrepresented minorities at NIH.

Complementary Publications:

1. Babahosseini et al. Unbalanced bidirectional radial stiffness gradients within the organ of Corti promoted by TRIOBP. *PNAS* 119 (2022). https://doi.org/10.1073/pnas.2115190119.

2. Efremov, Y.M., Cartagena-Rivera, A.X., Athamneh, A.I.M. et al. Mapping heterogeneity of cellular mechanics by multi-harmonic atomic force microscopy. *Nat Protoc* 13, 2200–2216 (2018). https://doi.org/10.1038/s41596-018-0031-8.

3. Cartagena-Rivera AX, Le Gal S, Richards K, Verpy E, Chadwick RS. Cochlear outer hair cell horizontal top connectors mediate mature stereocilia bundle mechanics. *Sci Adv.* 5(2):eaat9934 (2019). https://doi.org/10.1126/sciadv.aat9934.

Lab Website Section on Mechanobiology

Developing and applying advanced AFM methods that are specific to the requirements of different samples and exploring their three main biological questions are not the only focuses of this lab. Dr. Cartagena-Rivera said that a large amount of his time is spent writing reports and papers or giving seminars since he believes that communicating science both verbally and in writing is very important.

Learning How AFMs Work

Dr. Cartagena-Rivera had many years of schooling before he ended up as Chief of NIBIB's Section on Mechanobiology. However, he started out with experience in scanning electron microscopy, fluorescent microscopy, and microfluidics before learning how atomic force microscopy worked in Dr. Arvind Raman's lab as a graduate student. It was during the second year of his Ph.D. studies at Purdue when the first course was fully developed to formally train scientists at Purdue on the fundamentals of AFM.

Having a deeper/advanced understanding of how an AFM works is critical to continually developing what you can investigate with it, therefore this makes up one whole division of their lab. However, it is helpful to understand the fundamentals of this technology as described by Dr. Cartagena-Rivera.

> "An AFM fundamentally consists of a flexible cantilever that has different types of indenters at the end. It can have pointed tips, it can have spheres, or it can be also tipless. Basically, you use a cantilever and a laser detection system to measure the flexion or the deflection of the probe. So, what happens is that the cantilever is brought in very close proximity to the sample and then in contact, and when it's pushing against the sample, the cantilever bends, and that will change the spot or the location of the laser in a photodetector. That measurement, or shift in the spot detection location, is a measurement of how much the cantilever is being indented on the sample of interest."

Dr. Cartagena-Rivera's group is able to utilize this technology for advanced applications, such as "squishy physics," to look at the viscoelastic properties of different tissues and cells.

Exploring Fundamental Science Questions

Dr. Cartagena-Rivera and his group have a list of basic fundamental science questions that they are actively working on answering in the lab. These questions cover a variety of mechanobiology topics:

> "We are trying to understand the mechanical anisotropy in the sensory epithelium, which is the cochlea in the inner ear. We also have another project trying to understand the role of the actomyosin cytoskeleton and the glycocalyx, which is a modifying extracellular surface layer with mechanical properties in pancreatic cancer cells that will fuel aggression and cancer metastasis. Then, we have a third question that we're trying to understand that is actually divided into two things. One is on understanding how T lymphocytes are able to migrate efficiently through complex tumor microenvironments, and then the other part of the question is how the immune cells communicate to each other through mechanical signals."

Since there are various questions and topics they are exploring, they have to use a variety of AFM methods including multi-harmonic AFM, developed by Dr. Cartagena-Rivera during his Ph.D., for dynamic biological processes, such as cell crawling or migration.[2] For quantitative nanoscale viscoelastic properties, they use a different method developed by Bruker's BioAFM team called Quantitative Imaging Advanced mode. The last notable technique is what they use for sensorial tissue investigation, called noncontact frequency modulation AFM.[3] The investigation of tissue mechanics is the main objective of their lab and Dr. Cartagena-Rivera goes into detail about how the NIH equips them to conduct advanced experiments at this level;

> "Our lab has this expertise at the tissue level that not many other labs have. Since we are at the NIH, we have easy access to animal tissue and to human tissue. So, we can do this higher-risk complicated research here within the NIH, which would be more difficult or almost impossible in other institutions. So that's why my lab is now mainly focusing on tissue mechanics."

A lot of Dr. Cartagena-Rivera's work is being done to understand basic fundamental science and biological functions, but their investigation of tissues is also being used to help better understand cancer.

Tissue Investigation with Cancer Patients

Hot chemotherapy, or hyperthermic intraperitoneal chemotherapy, has been shown to increase the efficiency of chemotherapy treatment for patients. However, it is still not clear why it is working so Dr. Cartagena-Rivera and his lab are conducting research in collaboration with an NCI lab and the NIH Clinical Center next to their lab to probe these processes:

> "After they apply the hyperthermic intraperitoneal chemotherapy to the patient, the surgeon removes some of the treated tissue quickly and it gets to my lab from the clinical center in less than 20 minutes. Then, we quickly do atomic force microscopy to probe the mechanical properties of these tissues and we see that, after chemotherapy, there's a significant recovery, i.e., reduction, in the stiffness of these tissues. What is happening when we look in the tumor microenvironment using a fluorescent scope is that the collagen is being remodeled from being linear to being more curvy, as it was when the tissue was healthy. So definitely, the stiffness these collagen bundles provide is important for the progression of metastasis. By rebuilding this, you can hopefully enhance the efficiency of the chemotherapy because, in the stiffened state, these bundles can be a physical barrier for chemotherapy to penetrate."

A challenge that many research labs face is bringing their data out of the lab and into the real world, but Dr. Cartagena-Rivera believes the work they are doing has significant clinical relevance.

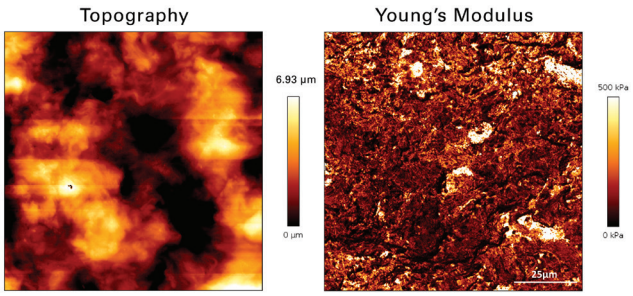


FIGURE 2.
Nanoscale topography and Young's modulus images of a human patient primary intratumoral pancreatic cancer after adjuvant therapy. The patient was resistant to adjuvant therapy and showed signs of disease relapse. These unpublished data are provided by Dr. Andrew Massey.

Bringing AFM Data from the Bench to the Bedside

Aside from the positive work they are doing to better understand cancer treatments, Dr. Cartagena-Rivera has various goals for using his research to bring accessible and equitable healthcare to all people. First and foremost, data collected from the clinic contains lots of information that needs to be analyzed while considering age, gender, ethnicity, etc. Additionally, where AFM data from the lab focuses on one cell or one tissue, that is not the case in the clinic. Therefore, the lab is working hard to try and use artificial intelligence (AI) and machine learning to deal with this heterogeneity and look at large datasets from AFMs in an unbiased way. Another goal that Dr. Cartagena-Rivera mentioned for further down the road was developing wearable devices that use AFM technology and are a point-of-care tool for people:

> "My dream is that we can design a miniaturized AFM-like device that you can wear. Imagine having something that has a small membrane, indenter, or something that can every so often push slightly on your skin to probe the local mechanical properties. For example, for skin diseases, when you have a rash and inflammation, you can touch it and you can notice that there are changes in stiffness. These are changes in mechanical properties the device would notice and may prompt you to visit your dermatologist."

Similar technology is already being adopted by many people, such as Apple Watches, which use laser-based detecting systems to give cardiac readouts. Next generation AFM-based wearable devices could have a profound effect in clinical settings for keeping patients safe by helping to catch diagnoses early. Overall, this lab group uses various AFM methods to obtain holistic measurements in a variety of biological systems.

Learn More

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