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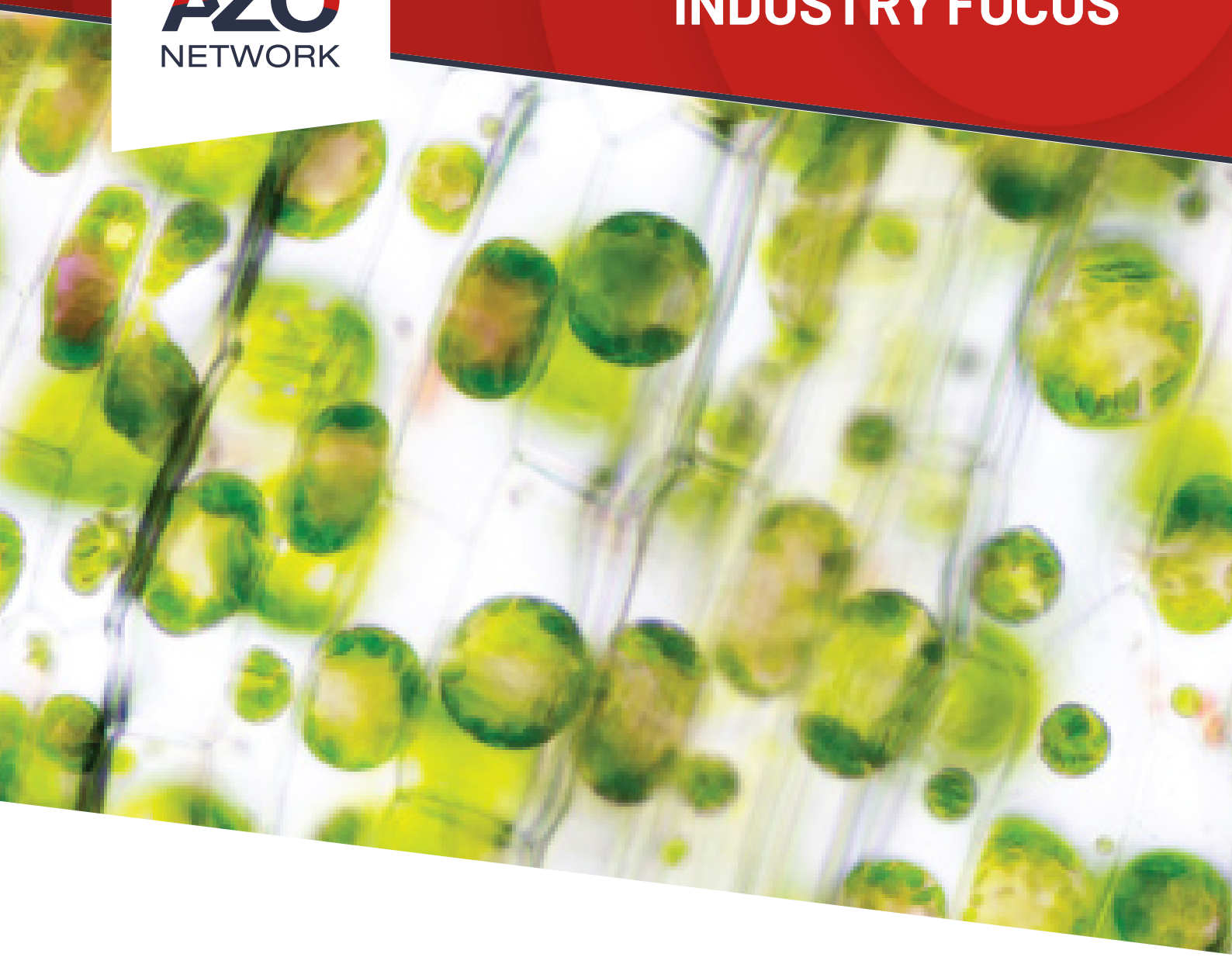
INDUSTRY FOCUS



Life Science Microscopy

An exclusive collection featuring top-tier articles, visionary experts, and essential industry insights

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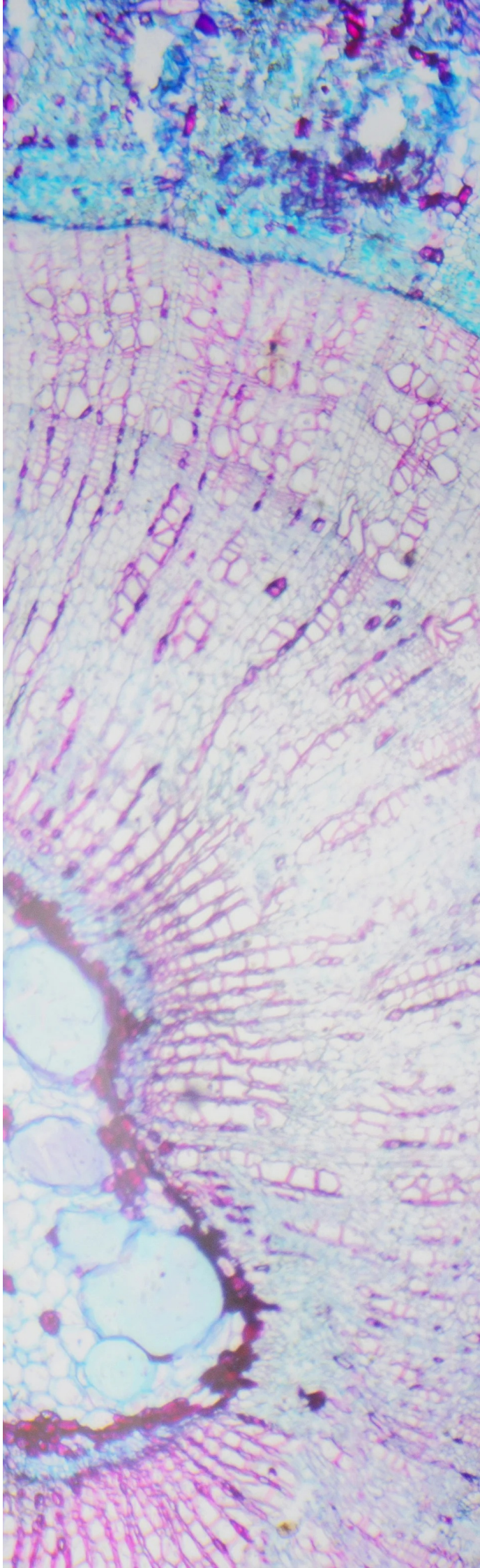
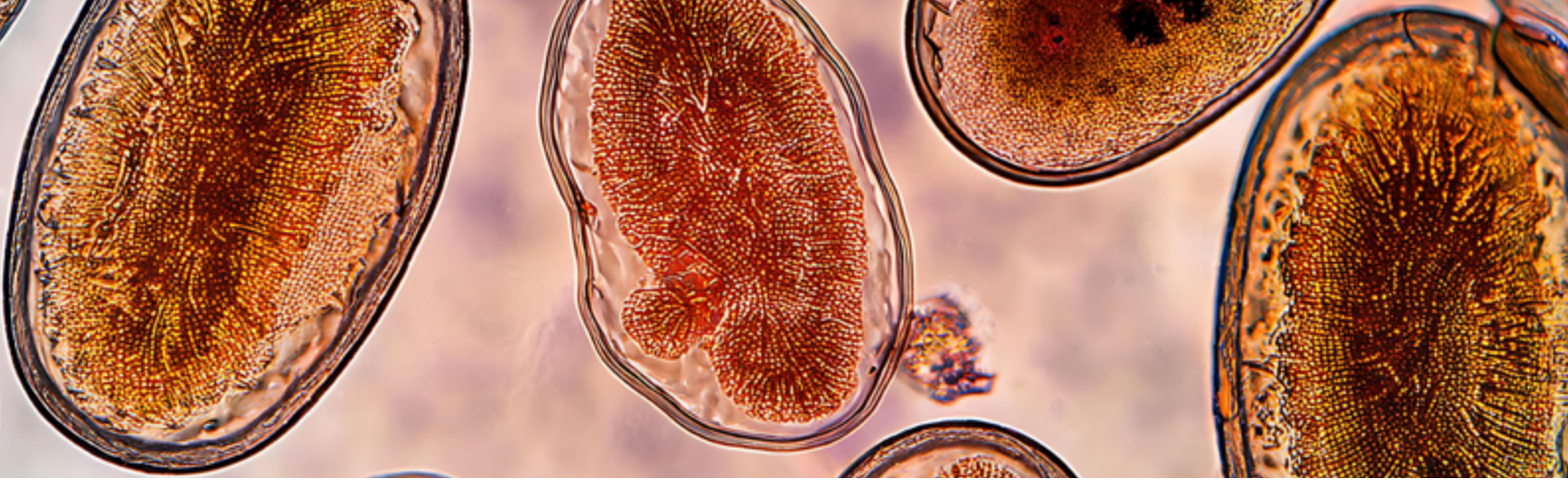




Foreword

Welcome to the latest edition of our Industry Focus eBook, where we explore the pivotal role of microscopy in advancing life science research. From unraveling the complexities of the nervous system to shedding light on viral replication and immune responses, microscopy continues to be a cornerstone of discovery in molecular biology, genetics, and cellular physiology.

Microscopy has revolutionized the way researchers visualize the inner workings of life. **Using Fluorescence Microscopy for Genetic Applications**, we examine how fluorescent markers enable unprecedented insight into gene function and cellular mechanisms. **Live-Cell Imaging with Super-Resolution Microscopy** furthers this, showcasing how researchers can now observe dynamic processes in living cells with exceptional clarity.

As neuroscience pushes into new frontiers, **Advances in Optical Methods for Systems Neuroscience** highlights cutting-edge tools illuminating how brain circuits function in real time. Meanwhile, in **How does norepinephrine influence anxiety-related behaviours?**, researchers leverage microscopy to investigate the neurochemical foundations of anxiety, offering potential avenues for novel therapies.

On the infectious disease front, **New Microscopy Reveals Secret of SARS-CoV-2 Replication** presents breakthrough findings that could inform future antiviral strategies. **The Discovery of Antibiotic Lolamicin that Targets Deadly Bacteria Without Harming Gut Microbiome** underscores how microscopy plays a vital role in antimicrobial research.

Microscopy is also transforming how we interpret cellular data. **Visualizing Cellular Data with Fluorescence Microscopy** delves into imaging techniques to decode complex cellular interactions. Meanwhile, **A Revolutionary RNA-based switch offers new control over gene expression in mammalian cells** and introduces a synthetic biology innovation that could reshape genetic engineering. Finally, **Salt can boost antitumor responses of T cells**, uncovers surprising immunological effects illuminated through advanced imaging.

Together, these articles underscore microscopy's diverse and transformative impact in the life sciences. We hope this eBook inspires new questions, deeper insights, and bold ideas as you continue your exploration into the invisible wonders of biology.

How does norepinephrine influence anxiety-related behaviours?

What are the roles of norepinephrine and the locus coeruleus (LC) in promoting anxiety-like behavior through the basolateral amygdala (BLA)?

The LC regulates arousal and stress reactions, especially by releasing norepinephrine. In the context of anxiety-like behaviors, norepinephrine release from the LC can influence neuronal activity in the BLA.

Our research has demonstrated that norepinephrine can increase the gain of neuronal ensembles in the BLA, which is especially important during times of stress or worry. This modulation effectively makes specific ensembles more sensitive, resulting in the anxiety-like responses we see.

Norepinephrine from the LC functions as a crucial neuromodulator, amplifying the effect of stress on the amygdala, a central center for processing emotional reactions.

Image Credits: luchschenF/Shutterstock.com

How do neurons in the amygdala form ensembles, and what is the significance of neuromodulation in controlling ensemble activity?

Neurons in the amygdala form ensembles that respond to certain stimuli—some respond to positive stimuli like sucrose, while others respond to negative stimuli like quinine. What is remarkable is how these ensembles work in opposition to one another.

For example, quinine can inhibit neurons that have been stimulated by sucrose and vice versa. We believe neuromodulation, particularly norepinephrine from the LC, is critical for controlling the activity of these ensembles.

This dynamic modulation scaling may help balance varying ensemble responses, regulating how an organism responds to both positive and negative stimuli. It could enable the brain to manage emotional reactions and behaviors in a context-dependent manner.

What challenges are associated with imaging and recording neuronal activity in the locus coeruleus, and how were these overcome?

The LC is a difficult region to work with for a variety of reasons. It is located deep in the brain and contains a tiny number of neurons, making imaging and recording difficult. One of the most difficult issues we faced was capturing stable recordings of neural activity while concurrently activating these neurons.

To solve this, we employed multi-photon microscopy in conjunction with advanced lens techniques such as prism lenses to penetrate deep into the brain and record neuronal activity. Combining these technologies has enabled us to both stably record as well as manipulate LC neuron activity in an awake and behaving mouse.

How does stress, such as exposure to predator odor, affect LC neuron activity, and what are the implications for norepinephrine release in the amygdala?

Stressors, such as the odor of a predator odor to a rodent, trigger synchronized activity in the LC, leading to significant norepinephrine release. We believe this synchronized LC activity amplifies the gain of neural ensembles downstream in the amygdala, heightening their sensitivity.

Our findings indicate that the increased norepinephrine release enhances anxiety-like behaviors by intensifying neuronal responses in the amygdala. During stress, the synchronized firing of LC neurons plays a crucial role in generating excessive emotional responses, further promoting an anxious state.

What effect does optogenetic stimulation of the LC-amygdala pathway have on anxiety-like behavior, and how is it modulated by norepinephrine receptors?

Using optogenetics, we were able to directly stimulate the LC-amygdala pathway and study its impact on anxiety-like behavior.

When we engaged in this route, we saw an increase in anxiety-like behaviors, which is consistent with the involvement of norepinephrine in the amygdala. Interestingly, pharmacological blockade of beta-adrenergic receptors caused a shift in how these neurons

responded to stress, while a specific genetic knock out of beta-2 adrenergic receptors lead to more active coping behaviors rather than passive anxiety-like responses.

This demonstrates that norepinephrine receptors, particularly beta-adrenergic receptors, play a vital role in how the brain processes stress and anxiety.

How do different neurons in the amygdala respond to stimuli of opposing valence (e.g., sucrose Vs. quinine), and what mechanisms underlie this antagonistic activity?

In the amygdala, neurons respond to both positive and negative stimuli, such as sucrose and quinine. We discovered that these neurons frequently exhibit opposite activity patterns; when one group is activated, the other is suppressed.

For example, when sucrose-responsive neurons are excited, quinine-responsive neurons become inhibited, and vice versa. This mutual inhibition implies a tightly regulated mechanism in which opposing ensembles interact to maintain emotional balance. We suspect that interneurons or other intermediate processes are mediating this antagonistic interaction, although we are actively exploring.

What insights do multi-photon microscopy and optogenetics provide in manipulating specific neuronal ensembles to alter behavior related to valence stimuli?

Multi-photon microscopy and optogenetics enable us to precisely target and control specific neuronal ensembles in the amygdala while also observing how these changes affect behavior.

For example, by stimulating neurons that respond to sucrose or quinine, we were able to manipulate mouse behavior in response to these stimuli. Specifically activating sucrose-responsive neurons enhanced the mice's likelihood of consuming a liquid it previously found unpleasent, whereas activating quinine-responsive neurons reduced positive actions, such as licking for sucrose.

These techniques offer amazing accuracy, allowing us to investigate how single neurons and their connections influence behavior.

How does synchronous activity in LC and amygdala neurons impact behavior, and what is the role of beta-adrenergic receptors in mediating these effects?

Anxiety-like actions are mostly driven by synchronous activity in LC and amygdala neurons. When the LC fires in a very synchronized manner, it causes a coordinated release of norepinephrine, which boosts the responsiveness of amygdale neurons. This increased neuronal synchronization improves emotional reactions, especially during stress.

Beta-adrenergic receptors, particularly the beta-2 subtype, are critical in mediating these actions. In tests where we shut down beta-2 receptors, we saw a decrease in anxiety-like behavior, indicating that these receptors are important in how the brain processes stress and regulates emotional responses.

About the Speaker

Sean completed his doctoral work in the laboratory of Dr. Susanne Ahmari at the University of Pittsburgh where he used mouse models to study the functional contributions of the orbitofrontal cortex and striatum in compulsive behavior. He is now a BRAIN Initiative K99 funded post-doctoral fellow at the University of Washington working in the laboratory of Dr. Michael Bruchas, where his work has demonstrated the causal relationship between discrete neuronal ensembles in the amygdala and valence-specific behavior.

Using Fluorescence Microscopy for Genetic Applications

Fluorescence microscopy is a workhouse technique in the life sciences for tissue analysis, cell structure visualization, and the study of biological processes and interactions.[1] More specifically for genetics research, typical uses of fluorescence microscopy include performing high throughput screening processes and studying the structure of genetic material.

Image Credit: Micha Weber/Shutterstock.com

What is Fluorescence Microscopy?

Fluorescence microscopy is an optical microscopy technique that works by detecting the emitted fluorescence from a sample. Normally, samples are labeled with a fluorescent tag known as a fluorophore – a chemical species that emits light on being photoexcited with a light source such as a laser.

Most standard optical microscopies illuminate a sample with white light and look at how much light is absorbed by different regions of the sample. The advantage of fluorescent techniques, particularly with advanced genetic modification methods, is that fluorophores can be used to label very specific parts of a genetic sequence or cell structure.[2] When dealing with large or

more complex samples, certain structures or regions can be selectively imaged – helping to deconvolute features that might be difficult to distinguish with standard transmission-based optical methods.

With a wide range of fluorophores now available, fluorescence microscopy can be a highly efficient technique for imaging multiple structures in a single experiment using optical filters.

If several cell sections are labeled with fluorophores that emit at different wavelengths, filters or selective excitation wavelengths can be used to only visualize one fluorescence wavelength at a time.[3] The information from different fluorophores can then be correlated to gain greater insights into the structure and, in particular, dynamical processes happening in the biological system.[4]

The development of readily available commercial solid-state lasers and microscopy platforms has helped the uptake of fluorescence microscopy in the life sciences considerably. Many companies offer all-in-one systems of the light source and microscope as well as acquisition software that can not just help with capturing images but with automated processes such as cell counting, cell structure labeling and Z-stacking – where images are taken at different focal distances and then summed together to try and reconstruct a 3D, rather than 2D image.

Some examples of fluorescence microscopy platforms include KEYENCE's instruments as well as long-established optics experts Leica, who offer a number of widefield fluorescence microscopy instruments. ThermoFischer Scientific offers all-in-one platforms like the EVOS M5000 imaging system that is designed specifically for use by cell imaging labs and even includes computer hardware such as an integrated monitor.

Greater automation capabilities also exist for microscope control and experiment design, with features such as automated focus finding helping improve the throughput of fluorescence microscopy platforms. Improved image quality and compatibility with advanced imaging stages, such as incubators, has enabled live cell imaging of cell growth and development.

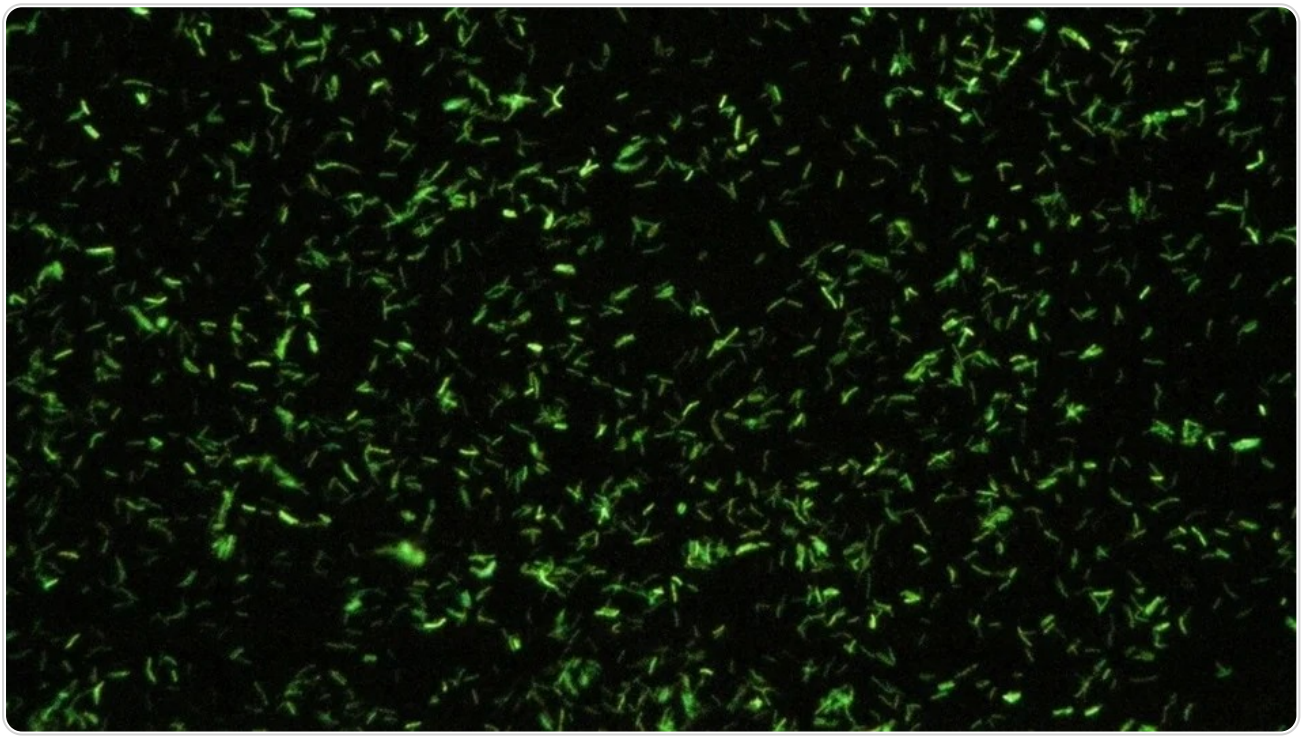
How Can Fluorescence Microscopy Be Used in Genetics Research?

One example of a genetics study that makes use of fluorescence microscopy is the use of time-lapse fluorescence microscopy to study gene regulation.[5] Time-lapse microscopy involves taking multiple images over a given time period to see how a process evolves as a function of time.

Time-lapse microscopy is very powerful in genetics research because the movement of the fluorescent signals can be used to study gene dynamics and movement, and the use of

particular fluorophore tags can also be used to look at properties such as inheritance. Depending on how the tag is created and attached to the species of interest, the tag may be transferred during processes such as cell differentiation and so the tag can be tracked as the biological process unfolds.

One of the advantages of using fluorescence microscopy for gene regulation studies is that it is a relatively non-intrusive method – particularly for fluorescent proteins that do not require the introduction of a fluorophore tag for imaging. Automation of processes, such as autofocusing, has also helped improve the quality of data that can be obtained through time-lapse methods.



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Challenges of Using Fluorescence Microscopy for Genetic Applications

The ability to introduce genetic mutations into proteins to introduce fluorescence and the availability of larger numbers of fluorescence tags have helped make fluorescence microscopy compatible with a larger number of chemical and biological systems.

However, finding good sample preparation procedures with some tags can be difficult and needing a tagged species can mean some small perturbations are introduced into the system.

As the fluorescence yield is dependent on the excitation intensity, it can seem reasonable to use higher average laser powers to perform fluorescence microscopy measurements. However, photodamage is a significant issue that limits the overall acquisition time of an

image.

The problem of photodamage and photodegradation is generally worse at higher laser powers and so it can be challenging to measure very weak fluorescence signals. This is also a problem for several electron microscopy approaches that generally offer better spatial resolution than optical microscopies but at the cost of more expensive instrumentation and greater problems with sample damage.

Fluorescent microscopy is a robust and versatile technique offering enhanced selectivity over standard confocal approaches. The development of superresolution methods is helping overcome the relatively large resolution limit set by the wavelength of visible light. More and more fluorophores are becoming available, making it possible to measure a greater number of systems.

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Further Reading

- All Genetics Content
- What is Recombinant Mapping?
- The Role of Genetic Regulation in Organogenesis
- Advancing Ancient DNA Research: New Methods Reshape Paleogenomics

Live-Cell Imaging with Super-Resolution Microscopy

Super-Resolution Microscopy transforms Live-Cell Imaging, offering unprecedented glimpses into cellular mechanisms with nanoscale precision. This cutting-edge technique is revolutionizing the understanding of biological processes, paving the way for breakthroughs in medical research.

Image Credit: Konstantin Kolosov/Shutterstock.com

Introduction to Super-Resolution Microscopy

The journey of microscopy began in the late 16th century with the invention of the compound microscope.[1] Over the centuries, scientists have been able to peer deeper into the world of the minuscule, revealing the complex structures of cells and microorganisms. However, the resolution of traditional light microscopy was limited by the diffraction limit, as described by Ernst Abbe.[2]

The advent of super-resolution techniques at the turn of the 21st century marked a revolutionary departure from this limitation. Techniques such as STED (Stimulated Emission Depletion)[3], PALM (Photoactivated Localization Microscopy)[4], and STORM (Stochastic Optical Reconstruction Microscopy)[5] have enabled scientists to observe structures at the nanoscale, far beyond the diffraction limit of light.

Super-resolution microscopy encompasses several techniques, but they all share the basic principle of circumventing the diffraction limit to achieve higher resolution. STED uses a de-excitation laser to minimize the area of fluorescence, allowing for imaging at a higher resolution.[3] PALM and STORM rely on the precise localization of individual fluorescent molecules that are switched on and off, building a composite image that reveals structures at a nanometer scale (10-30nm).[4,5]

The Leap to Live-Cell Imaging

Traditional microscopy techniques offer high resolution but cannot be used to image living cells. They involve fixing cells, which prevents capturing cell dynamics. In contrast, live-cell imaging studies cells in their growth medium, providing real-time spatio-temporal insights into cellular behavior.

This technique transforms snapshots into movies, visualizing and quantifying cellular processes over time. Observing cellular processes in real-time is crucial because it can reveal transient events that might be missed in end-point assays. It allows for the study of cellular structures in their native environment and provides insights into the dynamics and kinetics of cellular processes, such as protein interactions, trafficking, and signaling pathways, making them less prone to experimental artifacts.

Live-cell imaging has become an essential tool for comprehending dynamic molecular events in single cells and cellular networks.

Technological Advancements in Live-Cell Imaging

Recent technological advancements have significantly improved live-cell super-resolution imaging. Innovations such as improved fluorescent probes, faster and more sensitive cameras, and sophisticated computational algorithms have enabled researchers to image living cells with minimal disturbance to their natural state.

Recent advancements in super-resolution microscopy, particularly the MINFLUX technique, have revolutionized our ability to image at the nanoscale. MINFLUX combines minimal photon fluxes with standard fluorescence microscopy to achieve 1-3 nm 3D resolution, making molecule-scale imaging broadly accessible.[6,7]

Innovations such as synchronized beam steering and active-feedback stabilization allow for nanometer-precise localization of fluorophores in real-time. This method significantly outpaces traditional camera-based localization techniques like PALM/STORM, offering faster

tracking capabilities.[6,7] With these breakthroughs, MINFLUX has opened new avenues for investigating life sciences at the nanometric level.[6,7]

Other advancements in super-resolution microscopy involve the use of nanoprobes and photostable fluorescent nanomaterials.[8] These new probes have reduced inconveniences like photobleaching and phototoxicity associated with PALM, STORM, and STED. These innovations enable lower light power usage, enhanced photostability, and improved long-term imaging of live samples at high resolution.[8]

Case Studies: Breakthroughs in Cellular Biology

A good example of how Super-Resolution Microscopy is aiding in disease research is the study by Barabás et al. (2021).[9] The study focuses on neurotrophin receptors, specifically TrkA and p75NTR, which are vital for neuronal survival and are known to be altered in Alzheimer's disease (AD).[9]

By employing live-cell single-molecule imaging techniques, the researchers examined the behavior of these receptors on the surface of live neurons derived from human-induced pluripotent stem cells (hiPSCs) of familial AD (fAD) patients with presenilin 1 (PSEN1) mutations, as well as from non-demented control subjects.[9]

The findings demonstrate that the movement of TrkA and p75NTR on the cell surface and the activation of their related signaling pathways, specifically PI3K/AKT, is disrupted in neurons from fAD patients compared to controls.[9] This altered receptor trafficking suggests a potential mechanism contributing to the neuronal dysfunction observed in AD.[9]

The study underscores the importance of understanding receptor dynamics in disease conditions, which could lead to the development of new therapeutic approaches for AD.[9] The ability to observe these cellular processes in real-time has profound implications for disease research and drug development. It will help in the direct observation of the effects of pharmacological agents on cells, providing a powerful tool for drug screening and development.

Challenges and Considerations

Live-cell imaging is a complex technique that faces technical challenges such as maintaining cell health under artificial conditions, controlling the imaging environment, and minimizing phototoxicity and photobleaching.[10] Precise labeling of cellular components without affecting cell function, managing focus drift, and balancing imaging speed with resolution are also

critical.[10]

Additionally, the large data volumes require efficient management, and advanced microscopy techniques demand high sensitivity and expertise.[10] Overcoming these hurdles is essential for accurate real-time observation of cellular processes.[10]

Ethical considerations in live-cell imaging include ensuring informed consent for the use of human-derived cells, protecting donor privacy, managing data responsibly, respecting commercialization boundaries, and providing equitable access to personalized medicine.[11] Additionally, research must adhere to guidelines, especially when involving vulnerable populations or sensitive biological materials.[11]

Conclusion

Super-resolution microscopy has revolutionized live-cell imaging, offering nanoscale insights into cellular processes and driving advances in medical research. By enabling real-time visualization of dynamic cellular events, this technology has enhanced our understanding of disease mechanisms and facilitated the development of novel therapies.

Future prospects include overcoming technical challenges to improve cell viability and imaging quality and managing large data sets. As techniques like MINFLUX advance, it is expected that even more precise imaging will be developed, fostering breakthroughs in cellular biology and structural biology.

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Further Reading

- All Microscopy Content
- What is Microscopy?
- Microscopy and Artificial Intelligence
- Scanning Electron Microscopy (SEM): An Overview
- Investigating the Intersection between Microscopy and Healthcare

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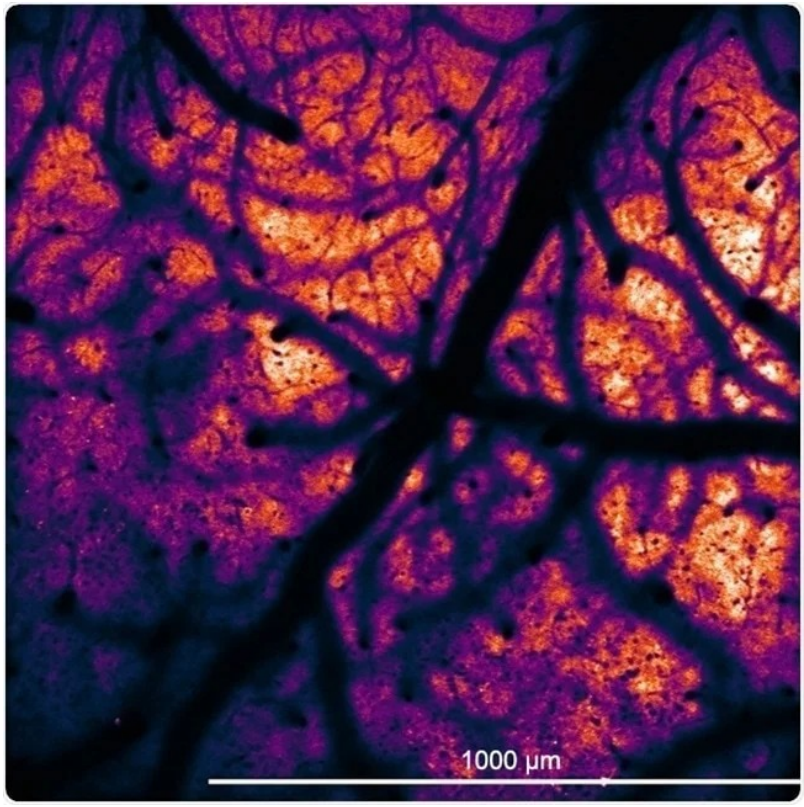
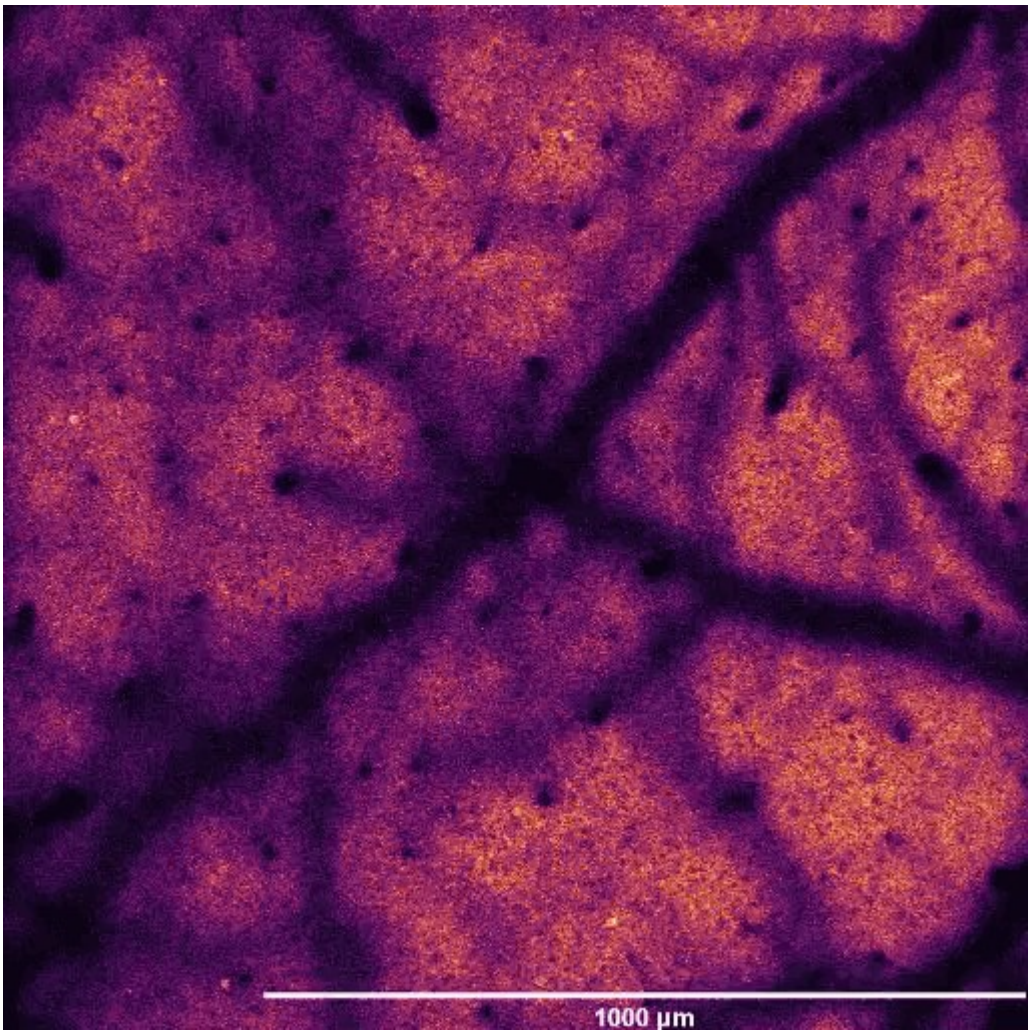
Innovation with Integrity

Advances in Optical Methods for Systems Neuroscience

Systems neuroscience research in recent years has been revolutionized by development of tools and techniques to aid in the understanding of neural circuits. Many of the more difficult questions relate to complex processes such as perception of stimuli, formation of memories in learning, or alterations in behavior based on social contexts.



Large FOV of GCaMP6 neurons in transgenic mouse.

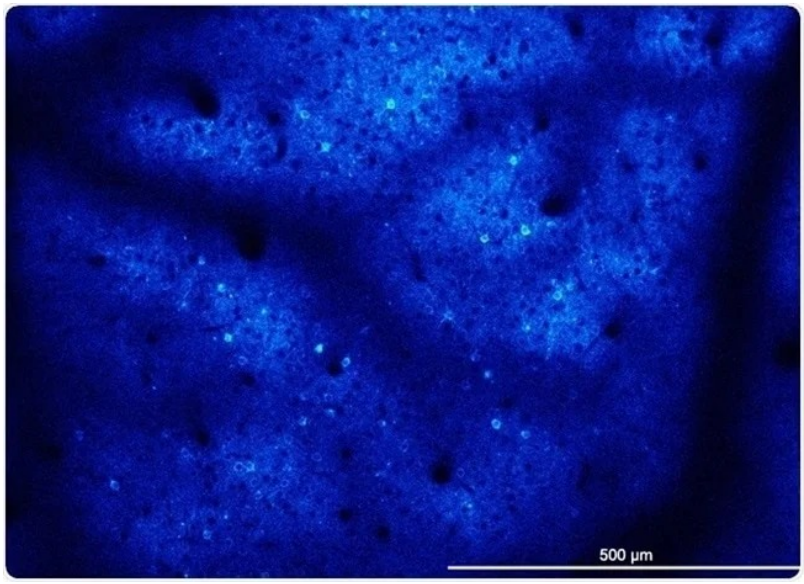


GCaMP6-expressing neurons firing in neocortex of a transgenic mouse captured at 15 fps. Image and video courtesy of Adam Packer, Robert Lees, and James Rowland (Packer lab, University of Oxford).

To study these higher-level problems, methods needed to be developed that were compatible with alive, awake, and behaving animal models.[1,2,3] One of the technologies that has been instrumental in this effort has been 2-photon or multiphoton fluorescence imaging. The adoption of 2-photon microscopy in neuroscience has allowed for in-vivo imaging deep into highly light-scattering tissue, such as within a mouse brain. Coupled with fluorescent probes, such as the family of genetically-encoded calcium indicators like GCaMPs, scientists could now image and record neural activity with cellular resolution in real-time.

More accurate measurement of neuron action potentials is becoming possible as fluorescent probes improve (such as with genetically-encoded voltage indicators). And as scientists begin to interrogate more complicated circuits, microscopy innovation also needs to advance. Fast xyz scanners and large field-of-view sizes, such as is possible with the Bruker Ultima 2Pplus multiphoton workstation, enable capture of circuit dynamics that span larger distances in the

brain. To prove hypotheses on the function of individual circuits, the advent of optogenetic probes,[4] allowing for light-activation of specific neurons, has been invaluable.



GCaMP6-expressing neurons in the neocortex of a transgenic mouse (JAX 024742). Courtesy of Adam Packer, Robert Lees, and James Rowland (Packer lab, University of Oxford).

Scientists (e.g., Packer et. al., 2015) have ingeniously used the channelrhodopsin probe and its 2-photon counterparts to induce activation of neural ensembles, demonstrating functional interactivity correlated with animal behavior.[1] This all-optical feedback loop of recording activity and stimulating neurons correlated to animal behavior prompted the collaborative development of spatial light modulator (SLM) stimulation technology, commercialized in the Bruker NeuraLight 3D. The SLM confers the capability to target multiple neurons in three dimensions by injecting a hologram pattern of light into the sample. Subsequently, after activation of one set of neurons, the SLM properties can quickly be changed through software command, and a different set of neurons can be targeted.

As tools like SLMs become more refined, techniques like optogenetics become more accessible, and closed-loop imaging and stimulation experiments will push forward our understanding of how neurons at the cellular level contribute to function at the system level.

Acknowledgements

This article was written by Jimmy Fong, Bruker Product Manager, Multiphoton Microscopes.

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New Microscopy Reveals Secrets of SARS-CoV-2 Replication

Recent research published in *Nature Communications* by a Stanford University team may improve drug development by examining at the nanoscale how the SARS-CoV-2 virus replicates in cells.

By using cutting-edge microscopy techniques, the researchers were able to capture what may be some of the clearest images of the virus's replication and RNA structures, which they saw forming spherical shapes around the infected cell's nucleus.

> *"We have not seen COVID infecting cells at this high resolution and known what we are looking at before. Being able to know what you are looking at with this high resolution over time is fundamentally helpful to virology and future virus research, including antiviral drug development."*
>
> *Stanley Qi, Associate Professor and Study Co-Senior Author, Department of Bioengineering, Schools of Engineering and Medicine, Stanford University*

Blinking RNA

The research sheds light on the molecular-level aspects of the virus's activity within host cells. Viruses take over cells and turn them into factories that produce new viruses, complete with unique replication organelles, to spread.

The viral RNA must repeatedly replicate itself within this factory until sufficient genetic material is accumulated to allow the virus to spread to other cells and recommence the process.

The goal of the Stanford researchers was to provide the most precise explanation of this replication step to date. They first used fluorescent molecules of various colors to label the replication-associated proteins and viral RNA to achieve this. However, a standard microscope would only show fuzzy blobs when imaging glowing RNA alone. Thus, a substance that momentarily suppresses the fluorescence was added.

The molecules would then sporadically blink again, only occasionally lighting up at a time. This made it simpler to identify the flashes and revealed the precise locations of each molecule.

Researchers captured images of the blinking molecules with a system comprising lasers, strong microscopes, and a camera that took pictures every 10 milliseconds. The researchers were able to produce incredibly detailed images that displayed the viral RNA and replication structures in the cells by combining sets of these images.

> *"We have highly sensitive and specific methods and also high resolution. You can see one viral molecule inside the cell."*
>
> *Leonid Andronov, Study Co-Lead Author and Chemistry Postdoctoral Scholar, Stanford University*

With a resolution of 10 nm, the resulting images provide what may be the most in-depth look at the virus's internal replication process to date. The pictures demonstrate how magenta RNA gathers around the cell nucleus to form clumps that eventually form a sizable repeating pattern.

> *"We are the first to find that viral genomic RNA forms distinct globular structures at high resolution."*
>
> *Mengting Han, Study Co-Lead Author and Postdoctoral Scholar, Stanford University*

The clusters help show how the virus evades the cell's defenses, said W. E. Moerner, the paper's Co-Senior Author and Harry S. Mosher Professor of Chemistry in the School of Humanities and Sciences. "*They're collected together inside a membrane that sequesters them from the rest of the cell, so that they're not attacked by the rest of the cell.*"

Nanoscale Drug Testing

Because the fluorescence labels blink, researchers can more accurately determine the location of virus components within a cell using this new imaging technique than they could with an electron microscope.

Additionally, it can provide nanoscale details of invisible cellular processes in medical research conducted through biochemical assays.

Moerner said, "*The conventional techniques are completely different from these spatial recordings of where the objects actually are in the cell, down to this much higher resolution. We have an advantage based on the fluorescent labeling because we know where our light is coming from.*"

Recognizing the virus's precise steps to infect a host has therapeutic potential. Seeing how various viruses infiltrate cells may provide light on why certain infections cause only minor symptoms while others can be fatal. Drug development can also benefit from super-resolution microscopy.

Han said, "*This nanoscale structure of the replication organelles can provide some new therapeutic targets for us. We can use this method to screen different drugs and see its influence on the nanoscale structure.*"

The team plans to repeat the experiment to observe any changes in viral structures caused by the presence of medications such as remdesivir or Paxlovid. The ability of a potential medication to inhibit the viral replication stage indicates that the drug is effective in hindering the pathogen and facilitating the host's defense against the infection.

The researchers also plan to map all 29 proteins that make up SARS-CoV-2 and see what those proteins do throughout an infection.

"*We hope that we will be prepared to really use these methods for the next challenge to quickly see what's going on inside and better understand it,*" said Qi.

Source:

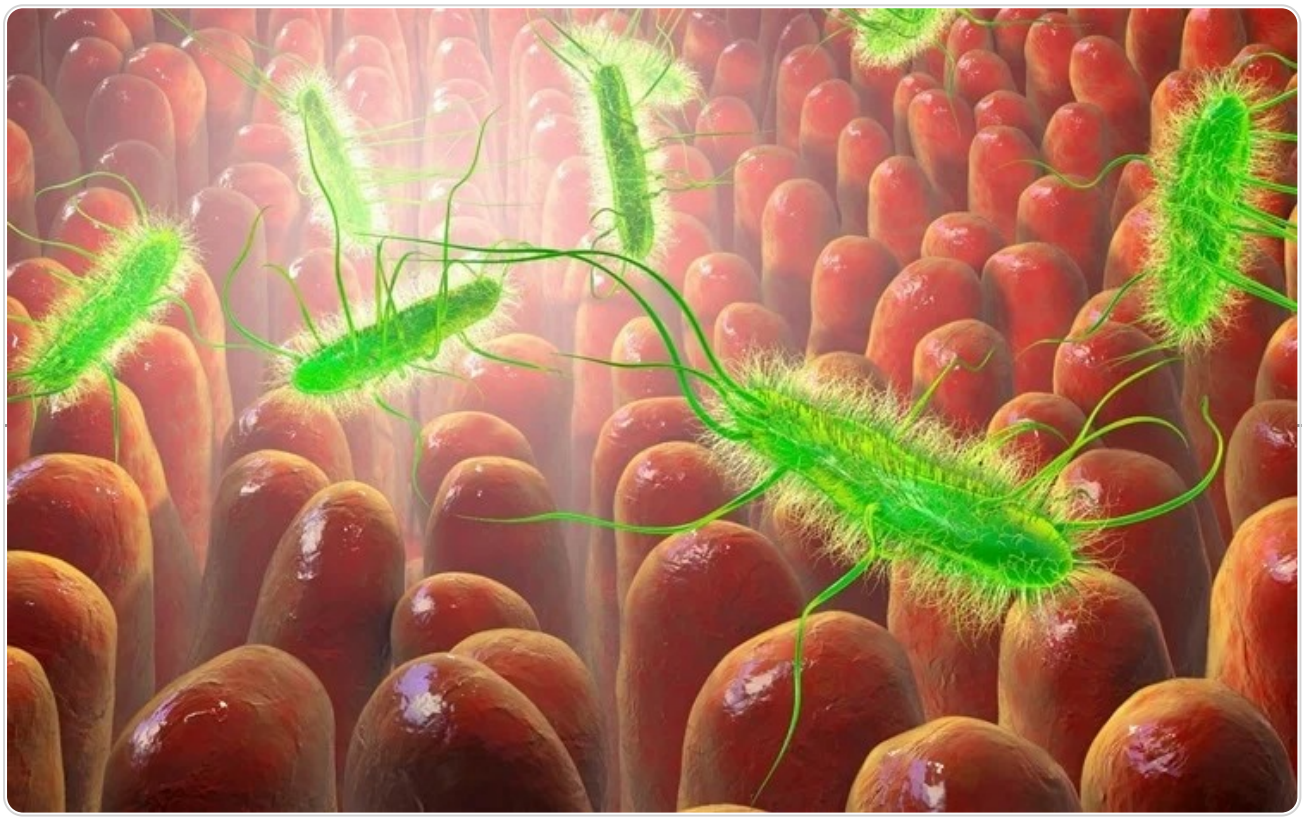
Stanford University

Journal reference:

Andronov, L., *et al.* (2024) Nanoscale cellular organization of viral RNA and proteins in SARS-CoV-2 replication organelles. *Nature Communications*. doi.org/10.1038/s41467-024-48991-x

Discovery of antibiotic lolamicin that targets deadly bacteria without harming gut microbiome

In a recent study published in the journal *Nature*, researchers in the United States of America designed and discovered lolamicin, a selective antibiotic that targets the lipoprotein transport system in Gram-negative bacteria. They found that lolamicin was effective against multidrug-resistant Gram-negative pathogens, showed efficacy in mouse infection models, spared the gut microbiome, and prevented secondary infections.



Study: A Gram-negative-selective antibiotic that spares the gut microbiome. Image Credit: Kateryna Kon / Shutterstock

Background

Antibiotic treatment can disrupt the gut microbiome, leading to increased susceptibility to pathogens like *C. difficile* and higher risks of gastrointestinal, renal, and hematological issues. Most antibiotics, whether Gram-positive-only or broad-spectrum, harm gut commensals and cause dysbiosis. The impact of Gram-negative-only antibiotics on the microbiome is unclear due to the scarcity of such compounds. Their discovery was challenging because most antibiotic targets are shared by both Gram-positive and Gram-negative bacteria. Since the gut

microbiome contains many Gram-negative bacteria, indiscriminate Gram-negative antibiotics such as colistin can cause significant dysbiosis, limiting their use.

Despite the rising need for new Gram-negative antibacterial agents due to resistant infections, no new class has been approved by the Food and Drug Administration (FDA) in over 50 years. Discovery is complicated by Gram-negative bacteria's complex membrane structures and efflux pumps. Developing a Gram-negative-only antibiotic that spares the microbiome calls for targeting an essential protein exclusive to Gram-negative bacteria, with significant homology differences between pathogenic and commensal bacteria. In the present study, researchers designed and reported a Gram-negative-only antibiotic named "lolamicin," that targets the Lol lipoprotein transport system in the periplasm, which is crucial for various Gram-negative pathogens.

About the study

In the present study, LolCDE, a key component of the Lol system in Gram-negative bacteria, was targeted. Screening was conducted for potential inhibitors of the system, which were synthesized and assessed. The efficacy of lolamicin was evaluated against multidrug-resistant clinical isolates of *E. coli*, *K. pneumoniae*, and *E. cloacae*. Susceptibility studies were conducted with lolamicin and other compounds.

Lolamicin-resistant mutants were developed and compared for fitness. The bactericidal effects of lolamicin were examined using time-kill growth curves. Confocal microscopy was used to observe phenotypic changes in the target bacteria. Molecular modeling and dynamics simulations, ensemble docking, and cluster analysis were used to explore lolamicin's binding sites and inhibition mechanism.

Further, mice were treated with pyridinepyrazole (compound 1) and lolamicin intraperitoneally for three days. Pharmacokinetic studies were conducted to assess lolamicin's bioavailability. Infection models were used to compare the efficacy of lolamicin and compound 1 in treating pneumonia and septicemia, with lolamicin also administered orally. Microbiomes of mice were analyzed using their fecal samples via16S ribosomal ribonucleic acid (RNA) sequencing. Additionally, antibiotic-treated mice were challenged with *C. difficile* to assess their ability to clear the pathogen spontaneously.

Results and discussion

Lolamicin, an inhibitor of the LolCDE complex, showed potent activity against specific Gram-

negative pathogens with low accumulation in *E. coli*. Lolamicin displayed selectivity, sparing both Gram-positive and Gram-negative commensal bacteria. It exhibited minimal toxicity towards mammalian cells and remained effective in the presence of human serum. Lolamicin demonstrated potent activity against multidrug-resistant clinical isolates of *E. coli*, *K. pneumoniae*, and *E. cloacae*. Lolamicin outperformed other compounds, showing a narrow minimum inhibitory concentration range and efficacy against multidrug-resistant strains.

Sequencing of lolCDE in resistant strains did not reveal mutations associated with lolamicin resistance, highlighting its potential as a promising antibiotic candidate. Lolamicin showed lower resistance frequencies across strains. LolC and LolE proteins were identified as targets, with specific mutations linked to resistance. Lolamicin exhibited bactericidal or bacteriostatic effects against tested bacteria. Swelling was observed in lolamicin-treated cells, indicative of dysfunctional lipoprotein trafficking. Lolamicin-resistant mutants displayed altered phenotypic responses to treatment, supporting LolC and LolE involvement.

Lolamicin was found to disrupt lipoprotein trafficking by competitively inhibiting binding at BS1 and BS2 sites. Hydrophobic interactions were primarily found to drive lolamicin binding, explaining the reduced efficacy of compounds with primary amines. Resistant mutations were found to impact lolamicin binding affinity, highlighting their role in destabilizing binding pockets. Lolamicin demonstrated superior efficacy to compound 1 in reducing bacterial burden and improving survival rates in infection models involving multidrug-resistant bacteria such as E. coli AR0349, *K. pneumoniae*, and *E. cloacae*.

Oral administration of lolamicin showed significant bioavailability and efficacy, reducing bacterial burden and increasing survival rates in mice infected with colistin-resistant *E. coli*. Lolamicin showed minimal impact on the gut microbiome with stable richness and diversity compared to amoxicillin and clindamycin. Lolamicin-treated mice and the vehicle control showed minimal *C. difficile* colonization. In contrast, amoxicillin or clindamycin-treated mice displayed an inability to clear *C. difficile*, with high colonization throughout the experiment.

Conclusion

In conclusion, this novel study identifies lolamicin as a pathogen-specific antibiotic that holds promise for minimizing damage to the gut microbiome and potentially preventing secondary infections. Further research and human studies are warranted to confirm the drug's clinical applicability. In the future, the microbiome-sparing effect of lolamicin could offer significant advantages over current broad-spectrum antibiotics in clinical practice, enhancing patient outcomes and overall health.

> *What if there was an antibiotic that doesn't disrupt the gut microbiome?*
> *There is now.*
> *A discovery published @Nature todayhttps://t.co/njtUddaJHo @PaulHergie and colleagues @UofIllinois @justsaysinmice pic.twitter.com/ONeFWYHEJL*
>
> *— Eric Topol (@EricTopol) May 29, 2024*

Journal reference:

- A Gram-negative-selective antibiotic that spares the gut microbiome. Muñoz, K.A. et al., *Nature* (2024), DOI: 10.1038/s41586-024-07502-0, https://www.nature.com/articles/s41586-024-07502-0

Visualizing cellular data with fluorescence microscopy

Fluorescence microscopy is commonly used in the study of biological systems mostly because of its specificity, ability to target structures individually, and its high signal over background. Recently, developments in fluorescence microscopy have allowed cellular detail to be observed at nanometer scales, much lower than the diffraction-limited resolutions of traditional procedures[1].

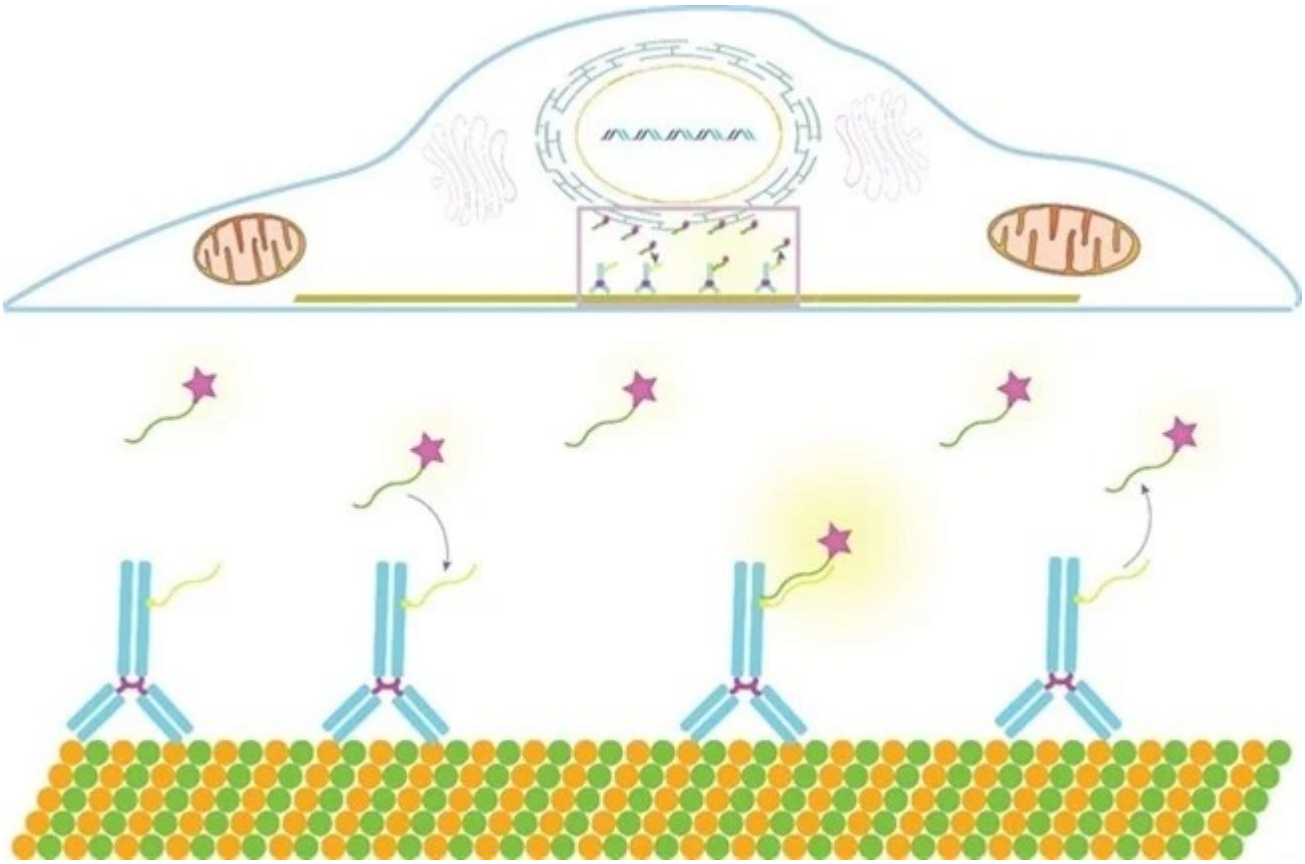


Figure 1. *How DNA-PAINT works. The target protein (tubulin) is labeled with an antibody tagged with the docking strand oligo. The sample is then bathed in imaging strand oligos. The transient binding of the fluorescently labeled imaging strand to the docking strand causes the sample to appear to blink, which can then be localized in the Vutara SRX software. Image Credit: Bruker Nano Surfaces and Metrology*

These developments steered the way into the field of super-resolution microscopy. This novel area has already had such a long-lasting influence on the community that two of its procedures were the subject of the 2014 Nobel Prize in Chemistry,[2-4] and instruments have become commercially available, like Bruker's Vutara 352 super-resolution microscope for single-molecule imaging.

However, these methods are not perfect. They are all faced with specific limitations and are

endlessly being developed to continue to grow their capabilities. For example, techniques that rely on stochastic switching of fluorophores, also known as single-molecule localization microscopy (SMLM, synonymous with STORM, dSTORM, PALM, etc.), face challenges with multicolor imaging using spectrally distinct fluorophores.

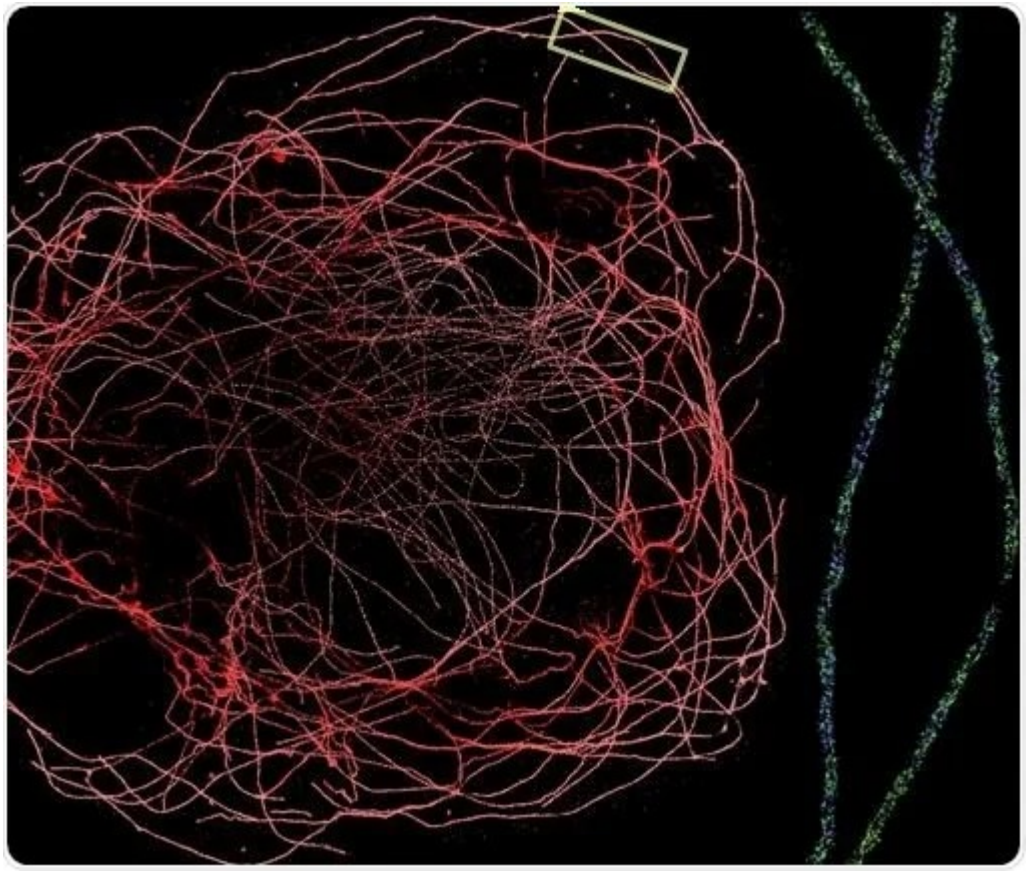


Figure 2. *DNA-PAINT allows sub-10 nm localization precision, making it one of the highest precision microscope techniques available. To the left is a DNA-PAINT experiment performed on the Vutara 352 microscope with a water immersion 1.2 NA objective. The image shows a whole BS-C-1 cell's tubulin network labeled with secondary antibodies conjugated to a DNA-PAINT secondary antibody. On the right is a zoomed-in section of the tubulin network (boxed region). The lumen of the microtubule is clearly visible. Image Credit: Bruker Nano Surfaces and Metrology*

This is mainly because of the lack of available fluorophores with desirable photo-switching properties[5]. A large amount of research has been completed to avoid this problem with new single molecule-based approaches.

One such approach that has been presented is DNA-PAINT (Points Accumulation for Imaging in Nanoscale Topography), which allows single-molecule localization through the binding of short (<10 nucleotides) oligonucleotides labeled with a fluorophore to a complementary oligonucleotide bound to a target molecule, usually an antibody or nanobody[6,7].

The short oligos binding is transient in nature, and thus forms a blinking effect akin to dSTORM or PALM, without the requirement for a unique photo-switchable fluorophore, as seen in Figure 1. DNA -PAINT has numerous benefits compared to alternative blinking techniques. The blinks usually last longer in comparison to traditional dSTORM, leading to higher photon yields from the fluorophore.

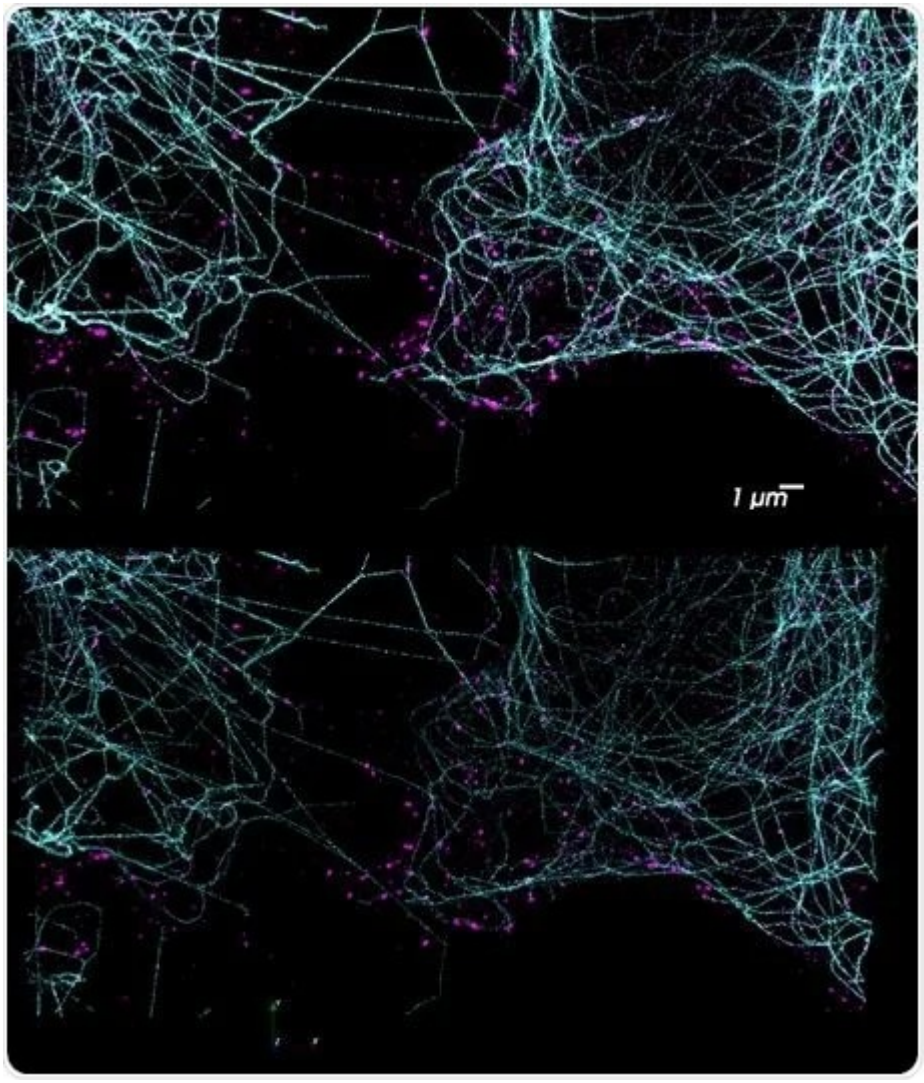


Figure 3. *DNA-PAINT has enormous potential for multiplexed imaging using the Vutara and its integrated fluidics unit. Using orthogonal docking strands on different probes, a potentially unlimited number of targets are possible. The top image shows a two-color DNA-PAINT experiment performed on the Vutara 352 single-molecule localization microscope. Tubulin is labeled in cyan and clathrin in magenta. Due to the unbleachable nature of DNA-PAINT, large Z-stacks are possible (bottom). Image Credit: Bruker Nano Surfaces and Metrology*

Consequently, it enables a much higher localization precision (<10 nm) in comparison to such methods as dSTORM and PALM. This can be seen in Figure 2. During imaging, the sample is bathed in an excess of fluorophore, enabling very long-lasting imaging, resulting in the signal being essentially unbleachable.

Target specificity is set by nucleotide sequence, so numerous targets are able to be labeled with different oligo sequences, enabling unlimited multiplexing potential with DNA-PAINT. Seeing these benefits, the PlexFlo Multiplexing Platform can wash the imaging strand for a given target from the sample, and different imaging strands labeling new biological targets can be added. This is shown in Figure 3.

Developments in methodologies like DNA-PAINT, in addition to advancements in commercially available tools and instrumentation, make employing these methods much more accessible for researchers.

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8. *Acknowledgements - This article was written by Lauren Gagnon, Fluorescence Microscopy Applications Scientist.*

Revolutionary RNA-based switch offers new control over gene expression in mammalian cells

In a recent study published in the journal *Nature Biotechnology*, researchers describe the pA regulator system based on a ribonucleic acid (RNA)-based switch to regulate mammalian gene expression by modulating a synthetic polyA signal (PAS) cleavage at a transgenic 5′ untranslated region (UTR).

Study: Control mammalian gene expression by modulation of polyA signal cleavage at 5′ UTR.
Image Credit: MMD Creative / Shutterstock.com

Current approaches to gene therapy

Gene control in mammalian cells is critical for developing safe and successful gene treatments. Current methods are associated with certain disadvantages, such as adverse immunological responses, limited efficiency, and therapeutic gene overexpression.

Current gene transfer technologies like adeno-associated viruses (AAVs) have difficulty performing conditional and reversible gene control. Toxic ligands, high leakage, and ligand

concentrations, as well as small dynamic range are some of the limitations associated with current RNA-based systems.

About the study

Luciferase assays were performed in mammalian cells, followed by RNA extraction from transfected cells for reverse transcription-polymerase chain reaction (RT-PCR) analysis. The researchers imaged stable cell lines by fluorescence microscopy and analyzed them by flow cytometry.

The AAV vector was created, followed by pA regulator-controlled luciferase expression experiments in mice and *in vivo* bioluminescent imaging. Additionally, pA regulator insertion was achieved by clustered regularly interspaced palindromic repeats (CRISPR)-associated protein 9 (Cas9) and fluorescence-activated cell sorting (FACS) of the regulator-controlled cluster of differentiation 133 (CD133) single-cell clones.

The researchers discovered ways to incorporate an artificial PAS into structured aptamers, which retained its activity, even when incorporated into structured aptamers. The synthetic PAS was managed by binding to Tc, a United States Food and Drug Administration (FDA)-approved oral medication. A synthetic PAS was added at regions where the cb32 aptamer sequence required slight substitution to form the PAS sequence 'AAUAAA' or in the single-stranded region.

Two polyadenylation-enhancing motifs were introduced to the synthetic PAS function. Luciferase activity in HEK-293T cells was measured to determine the cleavage efficiency of the synthesized PAS.

A6 was selected as the foundation for building the biological switch regulated by Tc due to its apparent high cleavage efficiency. The 'induction in fold' was calculated based on the proportion of luciferase signals in the presence or absence of Tc to assess Tc-induced expression. Taken together, over 180 constructions were created to systematically assess the impacts of various elements of the Y-shaped structure to improve the configuration of Y16.

A 'G-quad '-mediated and drug-inducible alternative splicing method was developed to manage the synthetic PAS to attain increased sensitivity and dynamic range. Several sequences that added a potential 3′ SS 'AG' downstream of the MAZ G-quad were also assessed.

Dose-response curves were generated using Y362 and Y387 as examples to define the complete regulatable range of the pA regulators. The pA regulator was assessed in live mice using an AAV2/9 vector encoding Y387 and the luciferase gene. To this end, mice were injected intraperitoneally with different dosages of Tc to evaluate the *in vivo* response of the Y387 pA regulator.

Study findings

The RNA-based switch pA regulator is a unique approach for regulating the expression of mammalian genes by modulating PAS cleavage. A dual mechanism regulates the cleavage triggered by drug binding and comprises aptamer fastening to restrict the cleavage of PAS and drug-elicited alternative splicing to remove the PAS.

Moreover, this technique avoids immunological reactions associated with other systems. As a result, an induction of 900-fold is achieved with a half-maximal effective concentration (EC_{50}) of 0.50 g/ml tetracycline (Tc), which is within the U.S. FDA-authorized dosage range.

The regulator could control luciferase transgenes in mice and CD133 expression by human tissues in a reversible and dose-dependent way, thereby ensuring long-term stability. The pA system enabled the generation of any intact protein as a transgenic product without changing its coding sequence, thus avoiding transgene-specific immune responses that have been observed in other systems. Moreover, the system had a 900-fold regulatable range while reducing baseline leakage expressions to 0.10%.

Tc-binding aptamers may influence gene expression by modulating PAS cleavage. The method involved inserting a synthetic polyA signal into a 5′ UTR, which caused efficient matching messenger RNA (mRNA) molecular cleavage. A small molecule-type ligand blocked cleavage by binding with RNA aptamer sequences, thereby retaining intact mRNA and inducing transgenic expression.

The Y-shaped structure of aptamers could inhibit PAS activity, whereas Tc binding enhances it at the G-quad. This would lead to downstream 3′ SS alternative splicing.

PAS and alternative splicing were strongly linked, and the pA regulator effectively regulated transgenes like the enhanced green fluorescent protein (eGFP). This system was efficient in multiple mammalian cell lines and is compatible with cells and promoters. Flow cytometry showed Tc recognition by the entire cell population, thus making it a 'portable' motif.

Conclusions

The study findings highlight the pA regulator system, an RNA-based switch that regulates gene expression by adjusting synthetic polyA signal cleavage in a transgenic 5′ UTR. This technique differs from traditional riboswitch systems, as the PAS is present in the 5′ UTR, combines the effects of numerous aptamers, and uses two processes of Tc binding and alternative splicing. However, the novel system can only use Tc as the inducer ligand, which cannot penetrate all body tissues efficiently.

Journal reference:

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Salt can boost antitumor responses of T cells

Sodium in tumor microenvironments found to heighten T-cell activation, suggesting new cancer treatment avenues

Study: Sodium chloride in the tumor microenvironment enhances T cell metabolic fitness and cytotoxicity. Image Credit: snezhana k/Shutterstock.com

In a recent study published in *Nature Immunology*, researchers investigated the direct influence of sodium (Na+) ions on the cytotoxic cluster of differentiation 8 (CD8)-expressing T cells and hence on antitumor cytotoxicity.

Background

The metabolic status of cytotoxic T cells or lymphocytes controls antitumor immunity. These cells are sensitive to the tumor microenvironment (TME). The TME can inhibit the anticancer immune responses by reducing T cell invasion, decreasing T cellular maintenance, and lowering effector activities. Studies indicate that extracellular ions such as potassium (K+) may influence T-cell functions.

K+ ions are abundant within the necrotic TME, inhibiting T-cell receptor (TCR)-driven effector functions while increasing stemness and multipotency. Elevated sodium ion concentrations stimulate T helper 17 (Th17) differentiation and increase self-regulatory cytokine expression.

However, the effects of sodium ion concentrations on cytotoxic T-cell-mediated antitumor immunity are unclear.

About the study

The researchers investigated the effect of sodium ion concentrations on cytotoxic T-cell activity and antitumor immunological responses.

Inductively coupled plasma optical emission spectrometry (ICP-OES) assessed K+ and Na+ concentrations in breast cancer and adjacent tissues. Researchers investigated the transcriptome imprint of sodium chloride exposure on cytotoxic T cells. They found that high NaCl treatment dramatically increased differentially expressed genes (DEGs), resulting in the sodium-chloride signature. They next investigated the enrichment of this signature in tumor tissues vs. healthy tissues. They also investigated transcriptome NaCl signature enrichment in cytotoxic T cells.

The researchers investigated the influence of NaCl on the transcriptome of human CD45RA-ve cytotoxic T cells expanded with CD3 and CD28 monoclonal antibodies (mAbs) in vitro. They analyzed transcriptome alterations using single-cell ribonucleic acid sequencing (scRNA-seq). They investigated the mammalian target of rapamycin (mTOR) signaling induction by TCR crosslinking of CD45RA–ve cytotoxic T cells through ribosomal subunit S6 phosphorylation under high and low NaCl circumstances.

The researchers investigated the molecular pathways by which increasing extracellular sodium-chloride concentrations can convert T cell receptor engagement in cytotoxic T lymphocytes into greater activation of T cells. They expected higher extracellular sodium chloride concentrations to enhance electromotive forces for Ca2+ entry following TCR-induced calcium (ORAI) channel activation. They investigated NaCl-induced relative hyperpolarization of membrane potential (or Vm).

The researchers created antigen-specific cytotoxic memory T lymphocytes by nucleofecting a melanoma-associated antigen recognized by T cells (MART-1)-specific TCR. They designed T lymphocytes that expressed second-generation chimeric antigen receptors (CAR) with 4-1BB-derived costimulatory domains that identified Panc02-mROR1 pancreatic cancer cells. They also investigated the effect of NaCl on CAR T lymphocytes bearing a CD28-derived costimulatory domain. Using a pancreatic cancer mouse model and the PancOva pancreatic cancer cell line, they tested whether NaCl increased T cell cytotoxicity during adoptive T cell therapy in vivo. Principal component analysis (PCA) and uniform manifold approximation and projection (UMAP) yielded the results.

Results

The study discovered elevated NaCl levels in breast cancer tissues, which resulted in transcriptional alterations in immune cells. Most tumor entities from the Cancer Genome Atlas (TCGA) have a transcriptomic sodium footprint compared to healthy tissues. The findings indicated that sodium is a relevant component of the tumor microenvironment. NaCl increased cytotoxic T-cell activation and activity, which improved metabolic fitness. Thus, NaCl enhances tumor cell death *in vitro* and *in vivo*. Elevated levels of genes like mTOR, tumor necrosis factor (TNF), interleukin-2 (IL-2), programmed cell death 1 (PD-1), mitogen-activated protein kinase (MAPK), interferon regulatory factor 4 (IRF4), and hypoxia-inducible factor 1 subunit alpha (HIF1A) corroborated these findings.

NaCl-induced alterations in cytotoxic T lymphocytes are associated with sodium-induced increased Na+/K+-adenosine triphosphatase (ATPase) activities and membrane hyperpolarization, which amplifies electromotive forces for TCR-induced downstream TCR signaling and Ca2+ influx. The adoptive transfer of cytotoxic T lymphocytes (CTLs) activated under high NaCl conditions effectively decreased tumor growth in a mouse pancreatic cancer model.

NaCl increased anticancer activity in the native T lymphocyte repertoire, CAR T lymphocytes, and transgenic ones. Under high NaCl circumstances, cytotoxic memory T cells increased the nuclear factor of activated T cell 5 (NFAT5) expression, resulting in prolonged survival in pancreatic cancer patients. Similarly, increased ATPase Na+/K+ transporting subunit alpha 1 (ATP1A1) gene expression was associated with pancreatic cancer survival in patients with Na+/K+-ATPase regulation. Researchers consequently argue that NaCl positively regulates acute antitumor immunological responses that may require adjustment to train therapeutic T lymphocytes like the CAR T variety, *ex vivo*.

Conclusion

The study showed that NaCl in the tumor microenvironment improves T-cell metabolic fitness and cytotoxicity. The mechanism of NaCl-induced T-cell hyperactivation includes internal Na+ translocation, increased Na+/K+-ATPase activity, membrane hyperpolarization, and higher electromotive forces for Ca2+ entry generated by TCR. Treatment strategies based on this mechanism may help cancer patients. Further clinical trials must evaluate NaCl's short- and long-term effects on antitumor immunity.

Journal reference:

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