Wiley Analytical Science
MAGAZINE

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Re-engineering nature to tackle climate change
– Profile of Ahmed Badran

A new storage concept with hydrogen production

Fuels from sunlight and air
– interview with Aldo Steinfeld

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Editorial

Dear Readers,

In a world marked by the pressing need for environmental sustainability, the latest issue of Wiley Analytical Science: Climate Solutions takes center stage, highlighting innovative and impactful solutions that stand at the forefront of the battle against climate change. As global temperatures continue to rise and the dire consequences of inaction become increasingly evident, our collective focus on climate solutions has never been more critical. In this issue, we explore groundbreaking research and developments aimed at mitigating the impacts of climate change and shaping a more sustainable future for our planet.

First, we explore the transformative work of Professor Ahmed Badran, a biomolecular engineer committed to re-engineering the ancient RuBisCO protein for more efficient carbon dioxide sequestration, offering a game-changing approach to global warming mitigation (page 19). Next, Paolo Gabrielli's interview illuminates the multifaceted challenge of decarbonizing the chemical industry, providing insights into various technological routes and their feasibility while highlighting the importance of regional contexts (page 24). Finally, we learn about a revolutionary thermochemical process developed by a research group at ETH Zurich, as they share how it can produce carbon-neutral transportation fuels from sunlight and air, charting a course toward the decarbonization of the aviation sector (page 37).

Addressing the climate crisis is no longer a matter of choice but an urgent necessity. The need for innovative solutions and a collective commitment to sustainability has never been clearer. The issue demonstrates the pivotal role that scientific research and technological advancements play in addressing the challenges posed by climate change. Together, we can pave the way for a more sustainable and environmentally responsible future. We hope the knowledge and insights shared in this issue will inspire action and foster a global commitment to climate solutions. The time to act is now, and the path to a more sustainable future is illuminated by the research and innovation discussed within these pages.

Would you like to contribute to our magazine? What would you like to read about? For suggestions on research topics and scientists you would like us to interview, please get in touch with us at AnalyticalScience@wiley.com.

Enjoy the read!

Dr. Cecilia Kruszynski
Editor, Wiley Analytical Science

Content

Wiley Analytical Science

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COVER STORY

Energy-efficient refrigerated circulators

Protecting the environment and lowering operating costs

Julabo
THE TEMPERATURE CONTROL COMPANY

Refrigerated circulators ensure reliable, accurate temperature control for applications involving samples, test specimens, and processes in, e.g., basic research, material testing, or technical systems. In many of these applications, partial load operation is usually sufficient for efficient heat withdrawal. Energy-efficient refrigerated circulators can help users save on operating costs while also reducing their environmental impact. When investing in new laboratory equipment, it is important to carefully verify the expected operating conditions for the desired systems so that the corresponding potential energy savings can be taken into account.

What does energy efficiency mean?

Generally speaking, energy-efficient products are used when attempting to achieve a specified target with the lowest possible energy use – in our case, precise temperature control of an application. The less energy needed, the more energy-efficient a product is. Products on the market with a similar function and performance can be used as a reference.

When can it be helpful to make use of energy-efficient systems?

With virtually all temperature control systems, a distinction can be made between the so-called base load and the usage-dependent energy consumption. The base load is required to operate the system. Usage-dependent consumption, on the other hand, results from the specific application and is affected by various factors, such as the required cooling capacity, ambient conditions, or type and number of connected consumers. The proportion of usage-dependent consumption is decisive for achieving greater energy efficiency.

To estimate the potential savings, the specific planned purpose of the systems must be considered. For many typical applications in the laboratory or industry, a fixed setpoint value is specified at the beginning of the temperature control process.

The temperature control instrument then tries to reach this setpoint in the shortest possible time. To do so, every component involved is operated under high load. Once the set point is reached, the desired temperature is usually just maintained. Here, it is sufficient for the components involved, e.g., fans or compressors, to operate at partial load. In these use cases, energy savings of up to 70% are possible when using energy-efficient temperature control systems. Conventional refrigerated circulators with a cooling capacity of less than 500 W are generally designed as capillary tube systems. These systems require comparatively little energy due to their low absolute cooling capacity. Using more expensive components, like electronic expansion valves and speed-controlled fans or compressors, to realize energy-saving potential would significantly raise the price of these systems.

At the same time, the overall effect in terms of energy savings would be very manageable due to their low cooling capacity.

In the past, JULABO has often used electronic expansion valves for refrigerated circulators with a cooling capacity of 600 W or more in order to realize energy savings, among other things.

The latest generation of refrigerated circulators is also equipped with a speed-controlled compressor and fan to provide greater savings potential. With the 800F and 1200F, the new refrigerated circulators are thus the first systems in their power range on the market that make use of this energy-saving potential.

How much energy can be saved: an example calculation

For this example, we will compare the MAGIO MS-1200F with the MAGIO MS-1000F.

The MAGIO MS-1000F has an electronic expansion valve. It is also equipped with a speed-controlled fan and compressor.

Operating point A:

The refrigerated circulator is temperature-controlled to the bath temperature specified in the table. In addition, energy is required to operate the pump. There is no additional load on the cooling machine (lower partial load range), and the temperature simply has to be maintained.

	Energy required in watt		
Bath temperature	20°C	0°C	-20°C
MAGIO-MS-1000F	927	878	861
Proportion, MAGIO circulator	435	375	375
Proportion, 1000F cooling machine	492	503	486
MAGIO MS-1200F	296	279	279
Proportion, MAGIO circulator	195	175	175
Proportion, 1200F cooling machine	101	104	104

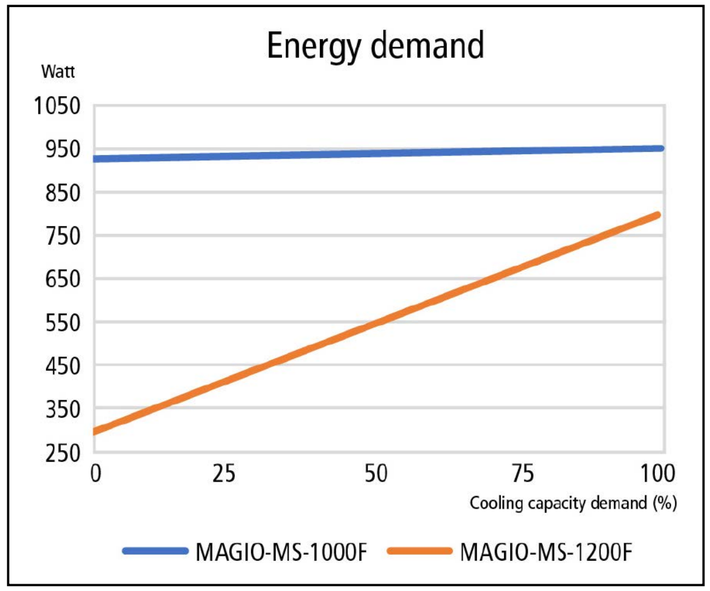
The result: In the lower partial load range, the energy required by the MAGIO MS-1200F is approx. 600 W less than the energy required by the MAGIO MS-1000F. The MAGIO circulator also has a lower proportion, since the 1200F further reduces the minimum cooling capacity, and less heating capacity is required for temperature control.

Operating point B:

The refrigerated circulator cools from +20°C to -20°C. There is an additional load requirement that ensures that the cooling machine cools at 100% power. The circulator does not require additional energy for temperature control. The only energy needed is for the pump.

	Energy required in watt
Bath temperature	20°C ... -20°C
MAGIO-MS-1000F	950
Proportion, MAGIO circulator	75
Proportion, 1000F cooling machine	875
MAGIO MS-1200F	800
Proportion, MAGIO circulator	75
Proportion, 1200F cooling machine	725

The result: Even if the cooling machine continuously cools at 100% power, the required energy can be reduced by 150 W.



Summary calculation examples:

The amount of energy saved varies depending on the cooling capacity demand. The lower the average cooling capacity demand is, the greater the potential energy savings will be. Because refrigerated circulators often do not cool continuously at 100% in practice, savings of 40-60% are typical.

Amortization time

With refrigerated circulators of 800 W or more, the energy savings result in rapid amortization of the initial additional costs. The cost savings result from the energy savings and the individual electricity price.

For our example calculation, we will again compare the MAGIO MS-1200F with the MAGIO MS-1000F. In this example, we assume that the application involves different temperature ramps and load requirements and that the partial load range levels out at approx. 50%. Based on the above example, the resulting energy savings would amount to 400 W/h. Since refrigerated circulators are used worldwide, various electricity prices are shown. The period of use is very individual. As such, we have provided several example calculations.

The example calculation shows that energy-efficient technology can be used to realize significant energy savings and reduce operating costs. Even with low electricity costs, these systems have an amortization time of less than 2 years in many use cases. At the same time, lower energy consumption means a positive contribution to climate protection.

Conclusion

Energy-efficient temperature control technology is perfect for many applications, especially with a cooling capacity of more than 800 W. The higher associated procurement costs are normally amortized within less than 2 years due to lower electricity costs. At the same time, users help to protect the environment by using energy-efficient refrigerated circulators.

Wiley Analytical Science

Wiley Analytical Science Award 2024

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Unveiling the future of MS with cutting-edge technology and AI

Exploring the AccuTOF GC-Alpha and msFineAnalysis AI for qualitative analysis

In this exclusive interview, we delve into the world of mass spectrometry and its innovative advancements. Our expert, John Dane, sheds light on the limitations of traditional EI mass spectral data and how the AccuTOF GC-Alpha, combined with msFineAnalysis AI software, is transforming the landscape of qualitative analysis, offering powerful solutions for identifying unknown compounds and addressing real-world challenges.

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The use of EI mass spectral data for library databases is common for qualitative analysis of GC-MS samples. Could you explain the limitations of EI as a hard ionization method and the challenges it poses when dealing with unknown substances?

J. Dane: EI, or electron ionization, is a high-energy ionization process that effectively bombards the molecule with high-energy electrons, causing it to break down in a consistent way. This consistency allows us to create databases like the NIST and Wiley databases, which are widely used. However, one inherent issue with EI is that it can fragment the molecule to the point where the molecular ion is no longer visible.

While it's common to see the molecular ions, it's also just as common not to see them. This can be problematic when multiple compounds share similar fragmentation patterns, making it challenging to identify the correct compound. Having molecular ion information can be extremely helpful.

In addition, library databases are limited to compounds that have been previously measured. For example, the Wiley and NIST databases cover around a million compounds combined, but the PubChem database lists over 100 million compounds. This limitation can pose challenges when working with real-world samples, as you're likely to encounter compounds that haven't been included in the databases.

How does the availability of various soft ionization methods (FI, PI, and CI) with the AccuTOF GC-Alpha assist in overcoming the limitations of EI mass spectra for identifying unknown components?

J. Dane: So, this actually hits upon what I mentioned in the first question. EI is a hard ionization process, so it's not surprising to find compounds that don't show their molecular ion. When you have soft ionization techniques like traditional chemical ionization (the most commonly available technique), photoionization (less common but simpler than CI), and field ionization (the softest technique), it becomes much easier to analyze these compounds due to the clear presence of molecular ion peaks. While not as commonly available, FI has been around for many years but was difficult to use on older sector instruments. More recently, time-of-flight systems have made this technique more accessible for GC-MS. Using any of these techniques combined with high-resolution mass spectrometry, you can generate a molecular ion (or adduct ions) and use the peak's accurate mass to determine a molecule's elemental composition. In turn, this molecular ion information helps narrow down the qualitative analysis results by using this information with the EI database search to help with identifying the compound.

It should be noted that not all soft ionization techniques produce the same results. Chemical ionization requires a reagent gas and involves reactions to produce molecular adduct ions like $[M+H]^+$, $[M+NH_4]^+$, etc. that can all appear at the same time, resulting in a more complicated mass spectrum. Photoionization is a simpler process that involves using photons to directly produce molecular ions and is especially useful for detecting aromatics. Lastly, field ionization effectively removes an electron from the molecules through electron tunneling to produce molecular ions even for saturated hydrocarbons, making it particularly suitable for the petroleum industry.

Dr. John Dane

John Dane graduated from Colorado School of Mines, US, in 2006 with a doctorate in applied chemistry with a focus on mass spectrometry. Then, he took a postdoctoral position at the National Renewable Energy Laboratory to study biodiesel combustion. John joined JEOL USA in 2008 as an MS applications chemist supporting all MS product lines, which include GC-MS, GCxGC-MS, MALDI-MS, and DART-MS. John was promoted to MS Product Manager in October 2020. He currently lives in the Boston area and enjoys getting outdoors whenever possible.

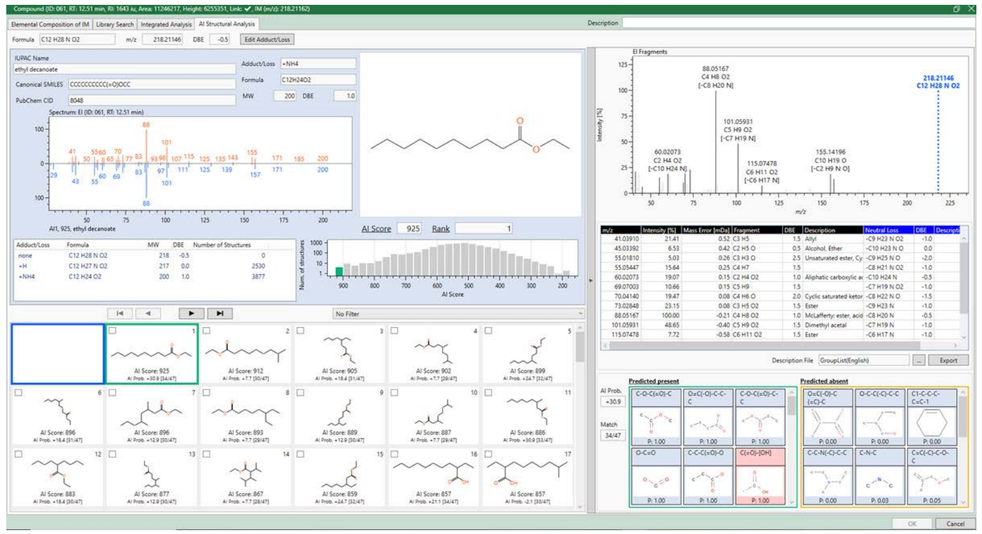
Could you describe the ion optics system of the JMS-T2000GC "AccuTOF GC-Alpha" and its role in achieving ultra-high resolution for the GC-TOFMS?

J. Dane: We basically took the previous generation system that had a 2-m flight path and we doubled it to 4 m. So it's 2 m up and 2 m down, so about 8 feet tall when you're talking about the overall height of the instrument. I always like to mention that the instrument flight tube comes disassembled, so you don't have to have a very tall door to get it into a lab. The bottom line is that by increasing the flight path, you allow those ions a longer time to travel, which allows them more time to separate from each other, thus resulting in a higher resolution system of 30,000 and a mass accuracy that is less than 1 ppm. Beyond that, we also retained all of our other capabilities including optional ion sources, data acquisition speed, and mass range from our previous systems. All of this together is very exciting as it makes our system extremely competitive with the other instruments out there.

What other advantages does the AccuTOF GC-Alpha offer over other systems out there in terms of data analysis?

J. Dane: Data analysis is one of the more difficult challenges to deal with as a mass spec analytical chemist as it is not uncommon to see hundreds of different peaks in a measured sample, which can be daunting to deal with manually. To address this situation, JEOL began offering a new software package called msFineAnalysis several years ago to handle the data processing automatically. This software combines all of the available information in the EI data and soft ionization data to help identify unknown compounds automatically.

In short, msFineAnalysis uses peak deconvolution and alignment, EI database searches, elemental composition calculations for each analyte using the soft ionization data, compares the calculated and measured isotope patterns, compares elemental composition candidates with the EI database matches to narrow down the possibilities, and then uses the EI fragment ion elemental compositions to further refine the results to the best possible candidate. This is all done automatically and results in a color-coded report for the analyst to prioritize their focus. Put simply, msFineAnal-



Examples of msFineAnalysis AI software.

ysis was developed to simplify the analyst's efforts in dealing with data.

This year, we released the 4th generation of this software msFineAnalysis AI. The latest version has artificial intelligence involved, which has been a significant leap forward in terms of qualitative analysis. When you measure a sample, it's common to see hundreds of different peaks, which can be daunting for analysts. With the introduction of AI, we addressed limitations in databases. We trained the AI using the NIST database, and it learned to map each molecule's structure, allowing it to correlate fragmentation patterns and expected fragments for different compounds. This AI was then used to create a predicted EI database for the more than 100 million compounds listed in PubChem and is provided with every copy of msFineAnalysis AI. This is not a cloud-based solution. It should be noted that the experimental data is still prioritized, with NIST or Wiley databases taking precedence. If those databases don't yield satisfactory results, the AI database comes into play.

Additionally, we've implemented a secondary AI on board the computer. It evaluates substructures and their coherence with the fragments observed, providing a 'yes or no' score. This simplifies the analyst's job by quickly assessing the molecule's build and substructure. While the user doesn't need to interact with it extensively during most of the process, it's a valuable tool for dealing with compounds not typically found in databases.

What are the key features and advancements offered by msFineAnalysis AI that differentiate it from the previous generation msFineAnalysis software?

J. Dane: This software is the first to utilize AI for qualitative data analysis in mass spectrometry. It represents our initial foray into this domain, and we're thrilled with its progress. msFineAnalysis AI has proven to be an exceptional software package, providing prompt and accurate results. What used to take 2-3 weeks for an analyst to work through, depending on sample complexity, can now be accomplished in less than an hour. It's an unparalleled time-saving tool.

Can you explain the working principle of the two newly developed AI models used in msFineAnalysis AI for structure analysis? How do these AI models help in providing candidate structural formulas, even for components not registered in the library database?

J. Dane: What the first AI is doing is giving you a 100 million compound database that you can compare against. Basically, it provides you with this information, which gets updated periodically by the factory. The second AI serves as a confirmatory substructure analysis tool. It looks at the compound itself and helps you decide what makes the most sense. You'll literally see a picture of the substructure's structure and compare it to the compound's structure.

How does the combination of the JMS-T2000GC AccuTOF GC-Alpha and msFineAnalysis AI software provide a powerful solution for the analysis and identification of real unknown compounds?

J. Dane: So that's where we've been pushing this system, especially for dealing with real unknown compounds. When you have these two components, the instrument and the software together, you get a complete package ideal for identifying true unknowns. The hardware provides access to the elemental compositions of the fragments and intact molecules, and the software automatically processes this information to help identify unknowns. You'll also have access to both soft and hard ionization. For soft ionization, you can pick from a menu of choices for the system. What's really cool is that we offer unique combination EI/FI/FD and EI/PI ion sources that have both soft and hard ionization. This means that you can switch between these methods without breaking the vacuum.

So it's crucial to have those combination sources that allow you to switch between soft and hard ionization because it avoids problems like having to loosen the GC column, which can lead to the column breaking, and your retention time changing?

J. Dane: Exactly. These combination sources are a crucial piece of the puzzle. With access to both hard and soft ionization, high resolution, and our software, analysts can work through the unknowns effectively.

We've also pyrolyzed polymers, like burning plastic, and most of the measured compounds created during this process were not found in the database. However, after processing the data with msFineAnalysis AI, the resulting unknown identifications all made sense when considering the polymer structure. None of these same unknowns would have been identified with typical hardware or software. However, we were able to identify many of these unknown compounds in just minutes. Having both the hardware and software capabilities together is very powerful.

Can you share some examples or success stories of how customers have utilized the msFineAnalysis AI and the AccuTOF GC-Alpha for challenging analytical tasks or identifying unknown compounds?

J. Dane: One aspect we focus on is materials analysis, particularly with polymers. Our msFineAnalysis AI software also includes a comparison feature, allowing you to analyze two different samples and pinpoint the variations between them. This is incredibly valuable for situations where you need to investigate issues, such as identifying problems with materials like good versus bad or old versus new. It can also be used for deformulation to uncover the composition of products or materials, offering a robust qualitative analysis capability to determine their constituents.

In summary, our system excels in fine chemicals and materials analysis, making it a powerful tool for analyzing, troubleshooting, and understanding the composition of various samples.

How does JEOL ensure the stability and reliability of the automated structure analysis function in msFineAnalysis AI, especially without the need for an online environment?

J. Dane: So, to clarify, the core AI predicted database cannot be currently updated by the user. If we discover the need to incorporate new compounds or update the AI algorithm in the future, we'll certainly make those additions and changes at that time. Looking ahead, there's the potential for individuals to register their own compounds, although this feature is not yet available, but it's on the horizon.

In essence, our approach revolves around continuously improving the AI's perspective. So, answering your question directly is a bit challenging because our system is dynamic, and we update it as needed.

Are there any plans for future enhancements or updates to the msFineAnalysis AI software or the AccuTOF GC-Alpha instrument that customers can look forward to?

J. Dane: That's a great question. We're continuously working on enhancing our hardware to improve its capabilities so this R&D is on-going. At the moment, there aren't any groundbreaking developments I can point to, but I can assure you that we're actively developing msFineAnalysis AI. We're planning to introduce new features that will facilitate database growth and provide better support for targeted scenarios, allowing you to find what you're looking for more efficiently.

I should also mention that our team will be meeting in Japan next week, where I'll gain more insights into our upcoming projects. So, while we're enthusiastic about our current offerings, there are certainly exciting developments on the horizon.

This interview was conducted by Dr. Cecilia Kruszynski, editor of Wiley Analytical Science.

At a glance:

Research updates on Climate Solutions

Earth's OZONE LAYER on the road to recovery

A United Nations report reveals that Earth's protective ozone layer is gradually healing, with the hole over Antarctica expected to fully mend in approximately 43 years. This recovery comes over three decades after the global ban on ozone-depleting chemicals, initiated by the 1987 Montreal Protocol. The report, presented at the American Meteorological Society convention in Denver, indicates that ozone levels are slowly improving, particularly in the upper stratosphere and the ozone hole.

Despite progress, the report notes that the global average of ozone in the atmosphere won't return to 1980 pre-thinning levels until around 2040 and even later for the Arctic. Antarctica's ozone hole is expected to recover fully by 2066. These findings demonstrate the success of international cooperation in addressing environmental challenges and set an example for addressing climate change.

Key factors include reduced levels of ozone-depleting chemicals, particularly chlorine and bromine, which have decreased significantly since their peaks. The Montreal Protocol's impact is evident in changing consumer habits, eliminating harmful substances from homes and cars, and ultimately preventing skin cancer cases. The report also highlights the potential danger of artificially cooling the planet using aerosols, which could further thin the ozone layer, particularly in Antarctica.

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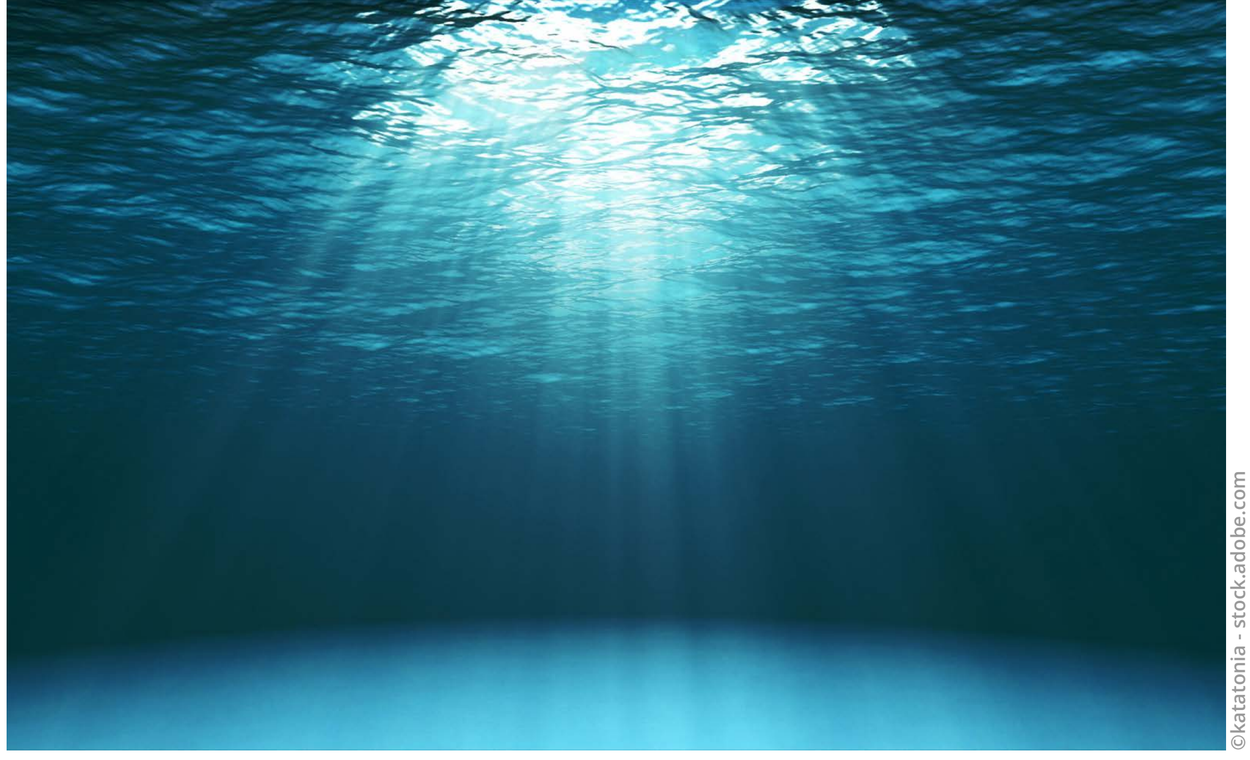

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Unprecedented GLOBAL OCEAN HEATWAVE threatens marine life and humanity

The world has witnessed an alarming rise in ocean temperatures, leading to a planet-wide ocean heatwave with dire consequences. June marked the highest-ever global ocean surface temperatures, setting local records from Ireland to Antarctica. Factors contributing to this phenomenon include climate change, weakened Sahara Desert winds, and the El Niño climate pattern. Leticia Carvalho, Head of Freshwater and Marine Ecosystems at the United Nations Environment Programme, emphasizes the devastating impacts of ocean heatwaves, including mass marine life mortalities, ocean acidification, disruptions to weather patterns, economic losses, and threats to global food security.

The frequency and intensity of marine heatwaves have doubled since the 1980s, according to a 2021 study by the UN's Intergovernmental Panel on Climate Change. Furthermore, hotter marine temperatures result in coral bleaching, algal blooms, and the displacement of marine species. With global emissions still on the rise, marine heatwaves are likely to become more common, endangering the ocean's vital role as a carbon sink and climate regulator. Urgent action is needed to reduce emissions, invest in nature-based solutions, and monitor and mitigate marine heat waves before they become even more catastrophic.

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The urgent need for INNOVATIVE COOLING SOLUTIONS in a warming world

July 2023 marked the hottest month in recorded history, highlighting the critical need for sustainable cooling solutions as traditional air conditioning exacerbates climate issues. Innovative technologies, such as separating dehumidification and cooling processes and harnessing evaporative cooling, offer promise for more energy-efficient and eco-friendly cooling systems. However, challenges, including costs and industry inertia, must be overcome to transition to these solutions. Ultimately, a cooler future must also incorporate passive strategies like urban planning and improved building design to reduce the demand for cooling.

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PLASTIC POLLUTION'S MINOR IMPACT
on climate change

Research findings reveal the limited impact of plastic pollution on climate change. While plastic production contributes to emissions, carbon leaching from existing plastics and incineration have relatively minor effects on atmospheric CO_2 levels compared to fossil fuel burning. The study suggests that addressing plastic pollution is essential for various environmental reasons, but its primary motivation should not be solely to reduce emissions.

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CLIMATE CHANGE and WINE QUALITY: How weather impacts European wine regions

European wine regions, from the UK to Sicily, are in the midst of their annual grape harvest. Researchers are studying how temperature and rainfall variations affect wine quality, with Bordeaux as a prime example. Warmer temperatures and specific rainfall patterns can lead to better wine quality, but climate change brings more extreme events, like hail and late frosts, which pose challenges to vineyards. Understanding these weather-wine links is crucial as the global climate changes, potentially reshaping the future of European wines.

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Journal insights

MIMICKING NATURE: Artificial reefs show promise in ecosystem restoration

In September 2023, Earth's average temperature exceeded the 1.5°C threshold, endangering coral reefs. Scientists are experimenting with artificial reefs made of concrete to replicate natural reef functions and support biodiversity. A recent study in Bali, Indonesia, shows promising signs that these structures can eventually mimic natural reefs, offering hope for restoring lost benefits in ecosystems affected by climate change.

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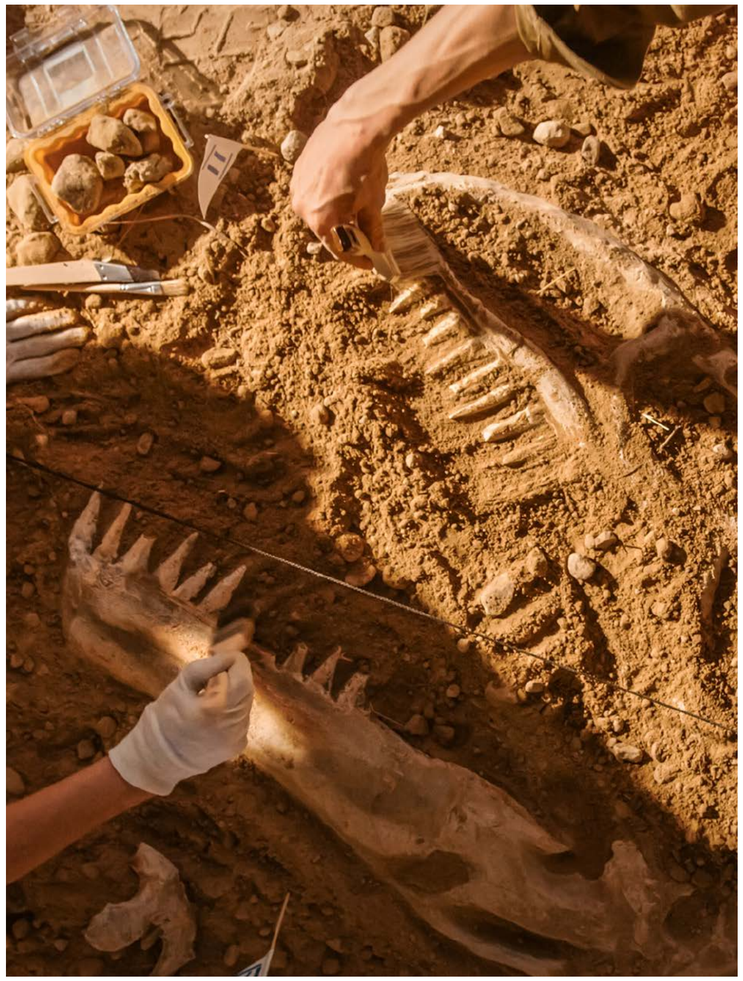

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ANCIENT MYSTERY unveiled

A recent study reveals that the Malvinoxhosan biota, a group of marine animals in the Early-Middle Devonian period near the South Pole, thrived during a time of global cooling but disappeared as the climate warmed. The research suggests that changes in sea levels and temperature were the primary factors behind their extinction, highlighting the vulnerability of polar ecosystems to environmental shifts. This ancient extinction event has implications for understanding modern biodiversity challenges.

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CLIMATE CHANGE threatens amphibian diversity in Brazil's Mata Atlântica

Researchers studied the impact of climate change on amphibian diversity in the endangered Mata Atlântica rainforest. Their research reveals that even moderate climate change trends could harm amphibians and the entire ecosystem, while real estate speculation in critical areas adds further pressure. To protect this unique ecosystem and its amphibian diversity, addressing climate change is crucial.

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Re-engineering nature to tackle climate change

An ancient and widespread protein, RuBisCO, holds huge potential for sequestering carbon dioxide, if only it was more efficient. Up-and-coming biomolecular engineer, Professor Ahmed Badran, believes he can now re-engineer the 'unevolvable' molecule and have a long-lasting impact on global warming – here's how.

To alleviate climate change, more and more scientists are turning to biology to find solutions, and Ahmed Badran is no exception. But what makes the Assistant Professor at The Scripps Research Institute a little different, is the high-risk research route he's taking. Right now, he is busy finding ways to bio-engineer RuBisCO, an ancient protein found in plants and algae that fixes carbon dioxide during photosynthesis and has the potential to remove vast volumes of the greenhouse gas, CO_2, from the atmosphere – if only it was a little more efficient.

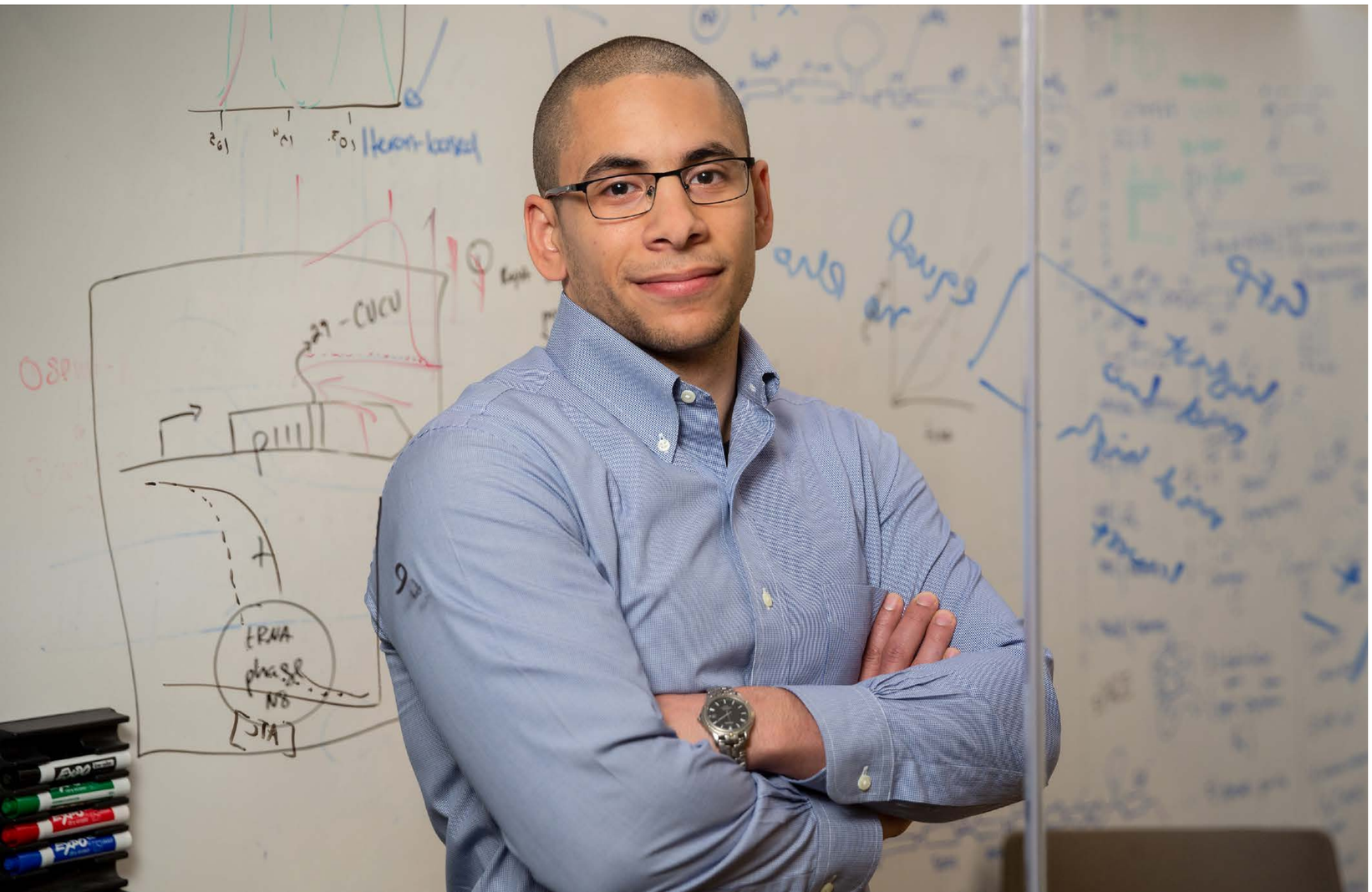
RuBisCO, otherwise known as Ribulose-1,5-bisphosphate carboxylase-oxygenase, is the most abundant carbon dioxide-fixing enzyme known to nature, yet it is remarkably inept at what nature created it to do. While a fast-acting enzyme such as carbonic anhydrase can capture a few million carbon dioxide molecules every second, RuBisCO typically captures the gas at somewhere between 1 and 22 molecules a second – which given its pervasiveness, is something of a shame.

Researchers far and wide have spent decades trying, and failing, to re-structure the enzyme's molecular features so it can catalyze carbon dioxide reactions more efficiently, but now Badran is optimistic he can change this. Since the age of 16, he has been working on the programmability of biology and has built numerous molecular tools to evolve better versions of proteins, including the formidable ribosome complex. RuBisCO is his new target, and he is "cautiously optimistic" of success.

"We're certainly taking a chance, it's hard to convince ourselves otherwise," he says. "But new engineering strategies can create biomolecules that nature has never seen. Goals that were impossible 10 years ago are becoming possible today."

Dabbling with DNA

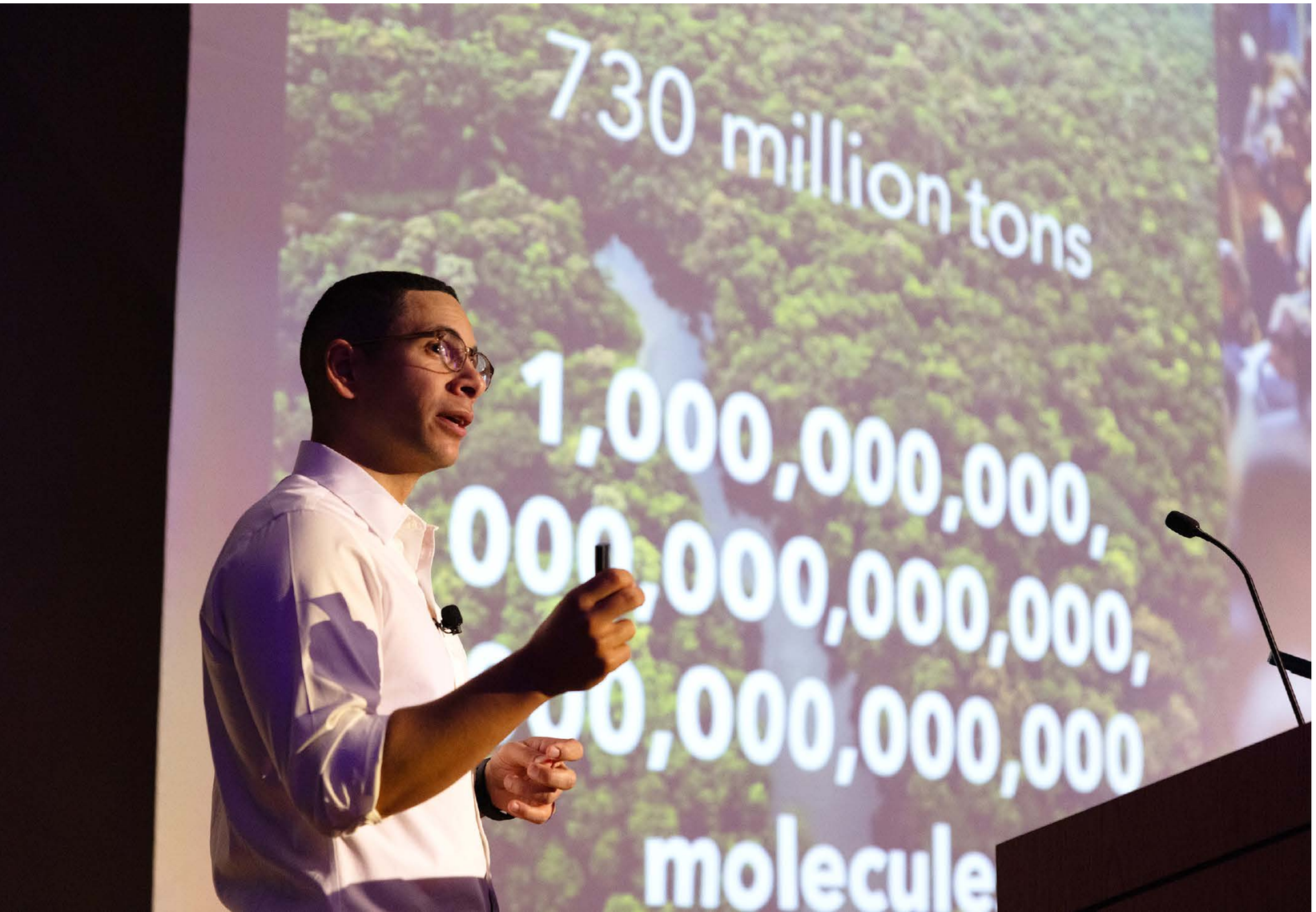
As the son of a botanist and astrophysicist, Badran was no stranger to science, but to this day, he remembers the impact of seeing green fluorescent protein in action for the first time. As part of an 11th-grade experiment at his high school in Arizona, Tucson High Magnet School, he delivered the jellyfish-derived gene to Escherichia coli bacteria growing in a Petri dish and later marveled at how the cells fluoresced under ultraviolet light. "This was a turning point for me," he says. "I saw the power of DNA and how it could code for a protein that absorbed and emitted light – it was foundational."



Ahmed Badran whilst at the Broad Institute, where he and colleagues were engineering ribosomes for protein production and antibiotic discovery. [Casey Atkins]

By this time, Badran was already recognized as an exceptionally gifted STEM student and was quickly paired with Professor Indraneel Ghosh, from Chemistry and Biochemistry at the University of Arizona, as part of the Southern Arizona Research, Science and Engineering Foundation (SARSEF) science program at his school. His future in biomolecules was sealed.

Ghosh was combining proteins from jellyfish, humans, and bacteria to design new biosensors, and Badran was fascinated. He worked in the Ghosh Lab up until he graduated from high school in 2006, and then as he says, "I wanted to stick around", so attended the University of Arizona as an undergraduate, continuing to work with Ghosh and taking on multiple degrees in Biochemistry, Molecular Biophysics, Molecular, and Cellular Biology. "This is how my path in science began," he says. "My research focus since then has been inspired by the incredible power and programmability of biology."



Proteins and ribosomes

Badran moved to Harvard University in 2010, to work in the lab of Professor David Liu on Directed Evolution. At the time, Liu was building molecular tools for rapid continuous directed evolution of proteins, including his breakthrough method, phage-assisted continuous evolution (PACE). This technique harnesses the 10-min life cycle of bacteriophages to rapidly evolve useful proteins.

By now, Badran had realized that protein bioengineering could sometimes fail for no clear reason, and wanted to explore evolutionary alternatives. "David Liu's group was developing a system capable of continuous directed evolution [PACE], which operated much faster than conventional approaches. I knew then that it would complement my bioengineering work and wanted to learn more," he says. "My time in the Liu Lab included some of the most exciting times of my career – the lab was highly enriched in exceptional scientists."

Badran spent a lot of time developing new capabilities in PACE, such as negative selection to steer protein evolution away from unwanted outcomes, and was instrumental in broadening the method's application from RNA polymerases to other protein systems.

Ahmed Badran, Assistant Professor of Chemistry at Scripps Research. [Scripps]

His tools – now considered fundamental to the field – have since been used in many projects, including evolving proteins to tackle bioinsecticide resistance, explore antiviral resistance, and also to improve genome editing tools and therapeutics.

“During my Ph.D., I learned there are two characteristics that define scientists,” he says. “We want to push the boundaries of human knowledge... and we need to communicate to the public that we can develop solutions to important problems.”

“The overlap of these two ideals can result in considerable impact – but one thing that 'sn't obvious is how often researchers attempt to do [all of this] and fail,” he adds. “For me, part of the learning process during my Ph.D. was to understand the right balance between the difficulty of a problem and whether or not it could potentially be solved.”

During his time with Liu, Badran also learned a lot about how to run a lab. “David always made time for us and was incredibly collegial,” he says. “I got to see the other side of the research paradigm – how to run a lab, write grants, and mentor students – important elements that define what it means to be a professor.”

Badran got the chance to start putting his experiences into practice when he was awarded a position as a fellow at the biomedical and genomic research center, the Broad Institute of MIT and Harvard, in 2017. As he puts it: “The opportunity to lead my own lab was a little too exciting not to take.”

The Broad Fellows program is designed to help early-career scientists establish independent research programs prior to accepting a faculty position, which is exactly what Badran did. “I had the best of both worlds as a Fellow,” says Badran. “This was a protected time to explore new and untested ideas, to be creative in my research program, and to push the boundaries of knowledge.”

Critically, with the fellowship in tow, Badran had the freedom to focus on higher-risk research than he might have been able to otherwise. So, with his small but ambitious lab group, he decided to use directed evolution to explore programmability in Nature's ancient and most intricate RNA and protein complex: The ribosome.

As part of their research, Badran and his team wanted to better understand how far programmability of the RNA-protein complex could be pushed, how they could alter translation, and how they could engineer it to make new proteins or change the way it reads the genetic code. They spent a lot of time evolving the ribosome as well as tRNAs, the molecules that carry amino acids to the ribosome during protein synthesis, and also learning, as Badran highlights, how to 'change the unchangeable'.

“How do you change what nature decided long ago is good enough?” he says. “We knew that if we could develop systems to do just that, we could push this complex and well-established molecular machine to do new things.”

Indeed, during their time at the Broad Institute, Badran's group used directed evolution to generate variations of ribosomal parts and tRNA molecules to build new proteins. In doing this, the researchers evolved ribosomes that could swiftly translate mRNA into proteins ready for developing biological medicines and also engineered E. coli to host ribosomes from different microorganisms for targeted drug development. Importantly, their successes cleared the path toward RuBisCO and defined the research direction in his lab more broadly.

"We became interested in biomolecules which, for reasons that remain largely unclear, were established very early in the history of life, and nature hasn't modified them much since," explains Badran. "The ribosome is a great example of this, yet we were able to show that it can be evolved to translate proteins nearly ten times better than what's found in nature."

"[This success] motivated us to explore RuBisCO for the same reason – we believe that if we can develop the right strategy, we can evolve it to be better at what it does," he adds.

Evolving RuBisCO

In 2021, Badran moved to Chemistry at The Scripps Research Institute, accepting the position of Assistant Professor. Here, his research has continued apace with his lab combining bioengineering, genome editing, and synthetic biology with directed evolution to develop antibiotics and biologics that bind to microbial ribosomes. "I've been very lucky. I was able to recruit excellent students and researchers who are ready to take on a challenge. We try to be judicious in how we pick these topics; they have to strike a balance between being a hard problem yet having enough evidence that they can be solved," he says.

And, of course, a further, key research strand is now to evolve RuBisCO to more effectively capture the greenhouse gas, carbon dioxide. Probably the most abundant protein on the planet, the carbon-fixing enzyme holds huge potential for tackling climate change, yet its slow rate renders it remarkably inefficient at this task. Many researchers have tried, and failed, to understand what limits its activity, so much so it has garnered a reputation of being 'unevolvable'. Badran and his team intend to change this.

A range of molecular approaches are already under development to help RuBisCO more efficiently fix carbon dioxide. For example, his group has developed new genetic code expansion technologies – which supplement the natural 20 amino acids of protein synthesis with new building blocks – to offer new evolutionary strategies towards more efficient carbon fixation. "We can integrate these building blocks into proteins with excellent efficiencies," says Badran.

The researchers have also devised metabolic engineering and ancestral protein reconstruction methods to better fine-tune RuBisCO and explore how its activity may have changed throughout its long, evolutionary record. "We're learning from these different processes – both the natural and engineered proteins that we can create – and believe we can distill all of this into a paradigm that makes RuBisCO and potentially other enzymes much more efficient," says Badran.

The Scripps Assistant Professor acknowledges that this task had turned out to be "quite complicated", but remains confident of the way forward, pointing to the continually growing RuBisCO knowledge base. "Thanks to innovations in related fields, we can now access DNA sequences and predict protein structure much more effectively," he says. "By bringing our modern technologies to this historically difficult problem, we can reasonably assume we'll learn something... and given everything we've seen so far, we're cautiously optimistic we can make RuBisCO better."

Indeed, in the near term, Badran also intends to re-engineer RuBisCO for more than climate change. For example, a more effective version of the natural enzyme could supercharge photosynthesis in crops, thereby raising plant growth rates and yields, while metabolites produced by RuBisCO catalysis reactions could also be salvaged and used to create biosynthetic drugs. Along the way, Badran is also clear on one point: he will enjoy the science.

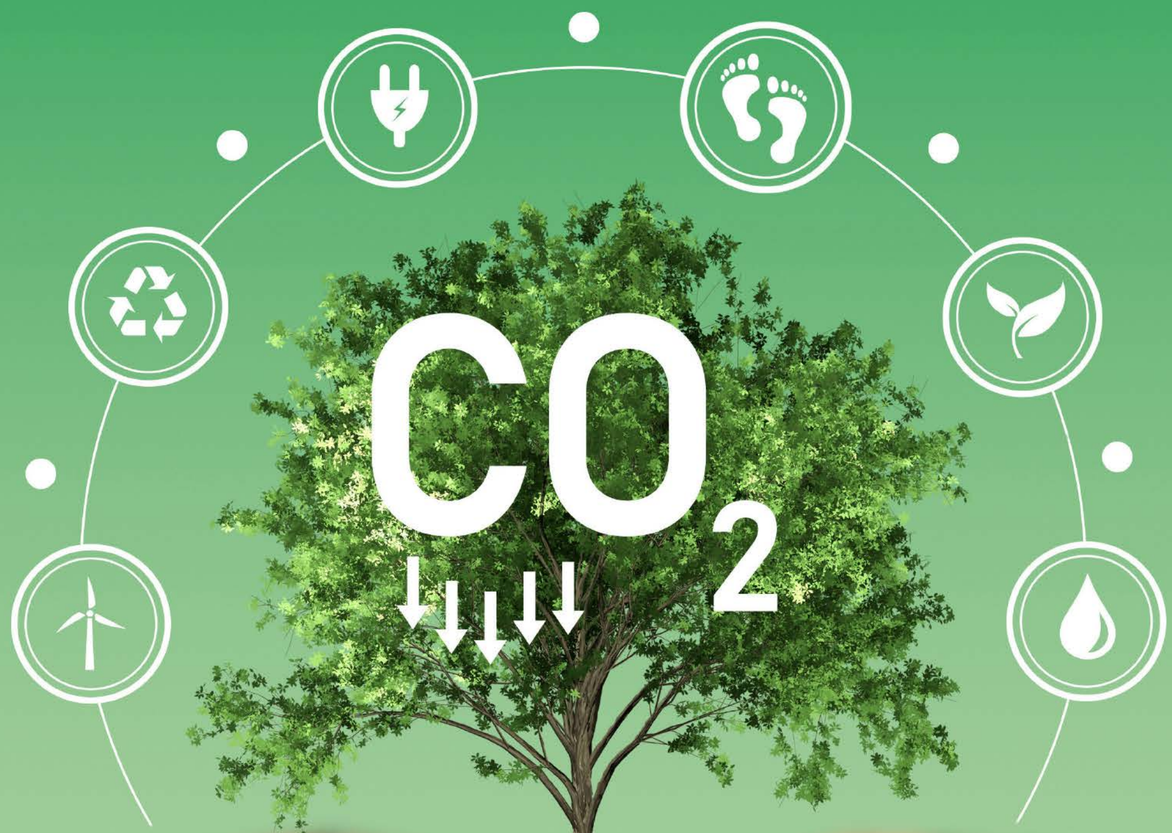
"When we pick hard problems, we are always cognizant that the failure rate will be high – this can make progressing through the research very difficult," he says. "But what has always pushed me, is the enjoyment I get from exploring the unknown – I enjoy the science and whatever path it takes us towards. Whatever the result, I'm confident it will be rewarding and fun."

This profile was conducted by Dr. Rebecca Pool.

Navigating the transition to a net-zero chemical industry

An in-depth interview with Paolo Gabrielli on sustainable chemical production

The chemical industry plays a pivotal role in our global economy, accounting for 5% of global CO_2 emissions and holding the key to achieving net-zero targets. In this insightful interview with Paolo Gabrielli, we delve into the multifaceted challenge of decarbonizing the chemical industry and transitioning to sustainable alternatives. We explore various technological routes, from carbon capture and utilization to biomass-based solutions, and discuss their feasibility and challenges. Gabrielli highlights the importance of considering regional contexts such as resource availability when crafting effective sustainability strategies for the chemical industry.



How can we effectively manage and transition from fossil-based raw materials to more sustainable alternatives as a net-zero society? How do these options differ and what factors influence their feasibility?

P. Gabrielli: The chemical industry is responsible for about 5% of global CO_2 emissions and is key to achieving net-zero targets. Decarbonizing this industry, however, faces particular challenges given the widespread use of carbon-rich raw materials, the need for high-temperature heat, and the complex global value chains. Yet, multiple technology routes are now available for producing chemicals with net-zero CO_2 emissions based on biomass, recycling, and carbon capture, utilization, and storage.

In the carbon capture and storage (CCS) route, chemicals are synthesized from fossil fuels as for business-as-usual (BAU) using the current organic chemistry. However, all CO_2 emissions generated along the chain are captured and permanently stored in suitable underground geological structures or in building materials. CO_2 can be captured from point-source emitters when available (such as refineries or ammonia production plants), via direct air capture (DAC), or a combination thereof. Overall, CCS routes are available today at a commercial scale. However, while considered key in abating the emissions of hard-to-abate industries, CCS routes rely on the continued use of fossil fuels and on the availability of large CO_2 storage capacity, which result in social acceptability challenges for CCS deployment.

In the carbon capture and utilization (CCU) route, the chemical industry achieves net-zero carbon emissions by substituting the provenience of carbon for C-based chemicals: From fossil carbon to the carbon in the CO_2, which has been previously captured from point-source emitters and/or from air. CCU requires the development of a new chemical industry, organic chemistry, and catalysts that convert CO_2 into the targeted C-based product, as well as low-carbon hydrogen and energy as inputs for product synthesis, as these are not extracted from fossil hydrocarbons any longer. Furthermore, CCU strategies would require the development and deployment of large CO_2 and hydrogen infrastructures to transport CO_2 and hydrogen from production to consumption sites.

Biomass contains both the carbon and hydrogen atoms as well as the energy required for the synthesis of chemical products. However, the chemical structure of biomass feedstocks is less favorable than that of fossil fuels, for example, in terms of higher water content and lower energy content. In the biomass route, CO_2 is captured from air via photosynthesis during the biomass growth and then emitted upon synthesis and end-of-life of the biomass-based product, thus resulting in net-zero CO_2 emissions. Whereas biomass is a promising feedstock for building a new chemical industry, this applies to several other sectors.

Dr. Paolo Gabrielli

Paolo Gabrielli is a Senior Scientist at ETH Zurich, where he studies the transition to net-zero emissions in energy systems and chemical production. He holds a B.Sc. and M.Sc. in Energy and Nuclear Engineering from the University of Bologna, and a Ph.D. from ETH Zurich, for which he was awarded the Hilti Prize 2021. Before joining ETH Zurich, he worked in the R&D division of General Electric Aviation and in the Renewable Energy Services practice of South Pole.

Therefore, its deployment might be hindered by its availability and by the land and water resources required to grow it. When available, residue biomass can be used as a feedstock without compromising available natural resources.

All routes can be coupled with processes that enable the use of waste and recycled material as a (partial) feedstock, as well as with demand-side measures to reduce the consumption of final products.

Could you provide insights into the current recycling rates of materials and potential strategies for further improvement?

P. Gabrielli: Carbon recycling applies mostly to plastic products. Today, only about 15% of plastic waste is collected for recycling at a global level; of that, 40% is discarded from the recycling process because of its low quality. As a result, average global plastic recycling rates are about 9%. With most plastic sent for recycling being downcycled because of its heterogeneous nature, both improved recycling technologies and collection processes are needed on the production and demand sides, respectively. Implementing effective recycling programs and incentivizing recycling can reduce the demand for virgin plastics. This includes creating markets for recycled materials.

What innovative strategies exist for curbing the demand for plastic products and promoting more eco-friendly alternatives?

P. Gabrielli: There are several innovative strategies for curbing the demand for plastic products and promoting more eco-friendly alternatives. These strategies often involve a combination of government policies, industry initiatives, and consumer awareness campaigns. Some key technology strategies include the following (see next question for policy strategies and initiatives):

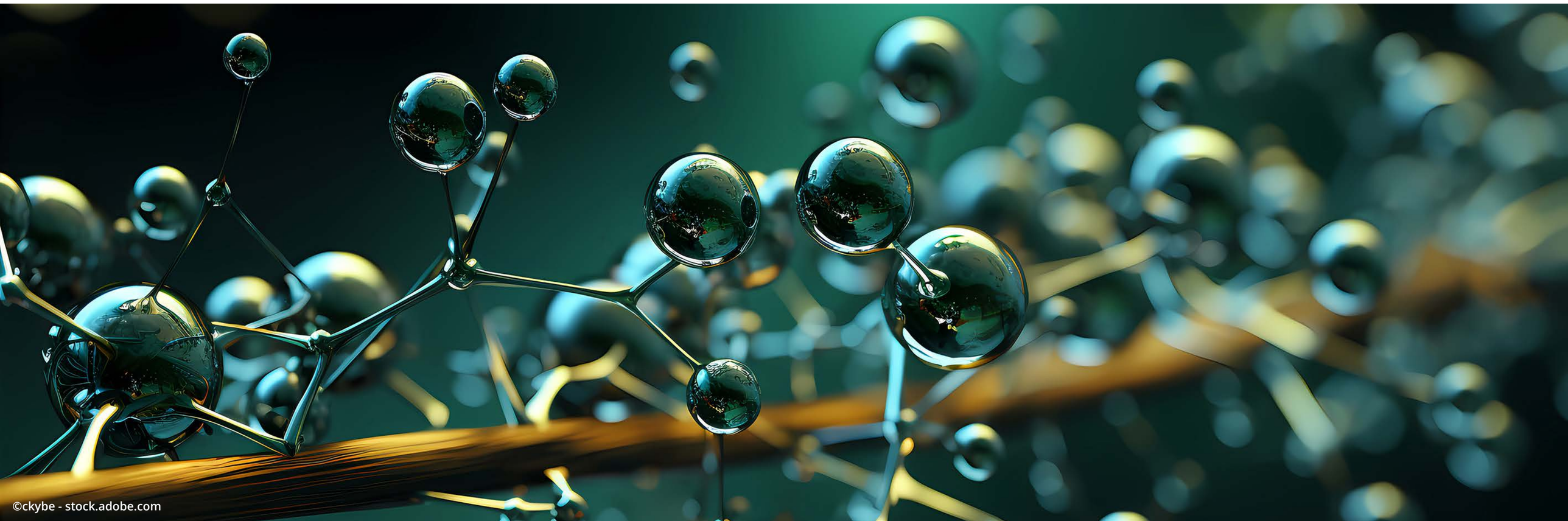
- **Eco-friendly packaging:** Companies are increasingly adopting innovative packaging solutions, such as compostable or biodegradable materials, edible packaging, and reusable packaging systems. This reduces the reliance on traditional plastic packaging and requires investing in research and development to develop new materials that are both eco-friendly and functional. This includes bioplastics, mycelium-based packaging, and other innovative materials.
- **Smart Packaging:** Smart packaging technologies incorporate sensors and indicators to monitor product freshness, quality, and usage. This reduces food waste and helps consumers make informed choices, ultimately decreasing the demand for excess packaging.
- **Edible Packaging:** Some companies are developing edible packaging made from materials like rice, seaweed, or potato starch. These packages can be consumed along with the food, eliminating the need for traditional packaging.
- **Innovative Business Models:** Some companies are adopting business models based on product-as-a-service, where customers subscribe to products and return them for refurbishment or recycling, reducing the need for new products.
- **Bioplastics and Bio-Based Materials (see biomass route above):** Researchers are developing bioplastics made from renewable sources like cornstarch, sugarcane, or algae. These materials can be used for various applications, from packaging to consumer goods, and are biodegradable or compostable.
- **Mycelium Packaging:** Mycelium, the root structure of fungi, can be grown into various shapes and used as a sustainable alternative to styrofoam and other packaging materials. It's biodegradable, lightweight, and can be customized for specific applications.
- **3D Printing with Sustainable Materials:** 3D printing technology allows for the creation of custom products using sustainable materials like recycled plastics, bioplastics, and even recycled ocean plastic, reducing the need for traditional manufacturing processes.
- **Plastics Recycling:** Advanced recycling technologies based on mechanical and chemical processes that can break down plastics into their molecular components, which can then be used to produce new plastics or other materials. This approach can help reduce plastic waste and reliance on fossil fuels for plastic production (see first question).

Continued investment in research and development in these and other areas can lead to more sustainable and eco-friendly alternatives to traditional plastics.

In practice, how can the various strategies for reducing demand be practically and sustainably integrated into our systems?

P. Gabrielli: I am not an expert on practical policy implementation, but I guess solutions include:

- **Plastic Bans, taxes, and restrictions:** Many governments have implemented bans or restrictions on single-use plastics, such as plastic bags, straws, and utensils. These measures encourage the use of alternative materials and reduce the demand for plastic products. An alternative could be taxes on plastic products, which can make them more expensive and discourage their use. Revenue generated from such taxes can be used to fund environmental initiatives.
- **Subsidies and incentives:** Governments can offer subsidies or tax incentives to businesses that use eco-friendly materials or adopt sustainable practices. This encourages the adoption of alternatives to plastic.
- **Circular economy initiatives:** Promoting a circular economy involves designing products for reuse, recycling, and repurposing. This reduces the demand for new plastics by extending the life of existing materials.
- **Consumer education and awareness:** Public awareness campaigns can help educate consumers about the environmental impact of plastic and encourage them to make more sustainable choices. Social pressure can also drive change.
- **Collaboration and partnerships:** Collaboration between governments, businesses, and non-governmental organizations (NGOs) can lead to more effective strategies for reducing plastic demand and promoting alternatives.
- **Extended producer responsibility (EPR):** EPR programs make manufacturers responsible for the entire lifecycle of their products, including disposal and recycling. This incentivizes them to use more sustainable materials and reduce plastic use.
- **Lobbying and advocacy:** Environmental organizations and concerned citizens can lobby for policy changes and advocate for more sustainable practices in industries that heavily rely on plastic.



These strategies are often most effective when implemented together as part of a comprehensive approach to reduce plastic demand and promote eco-friendly alternatives. Additionally, progress in this area requires ongoing research, policy adaptation, and collaboration between various stakeholders.

What are the viable avenues for extending the lifespan of products through reuse and what challenges might need to be addressed?

P. Gabrielli: There are several viable avenues for extending the lifespan of chemical products, but they come with their own set of challenges that need to be addressed. Here are some key avenues and associated challenges:

- **Refill and reuse systems:** Implementing refill and reuse systems for products such as cleaning agents, personal care products, and detergents. Customers purchase a durable container and refill it with bulk products. Challenges associated with this might be (i) consumer behavior: Encouraging consumers to adopt refill and reuse habits can be challenging due to convenience and habit issues, and (ii) logistics: Establishing collection and refill points and maintaining a clean and efficient system can be complex.
- **Chemical recycling:** Chemical recycling technologies can break down chemicals and plastics into their base components, which can then be used to produce new products. However, developing and scaling up chemical recycling processes can be technically challenging and costly.
- **Remanufacturing:** Remanufacturing involves refurbishing and repairing chemical products, such as industrial equipment, to extend their functional life. However, this requires developing uniform standards for remanufactured products to meet safety and quality requirements. Moreover, managing the supply chain for remanufactured products can be complex and require collaboration with multiple stakeholders.
- **Sharing platforms:** Sharing platforms enable businesses to share chemicals and resources among themselves, reducing the need for individual ownership. Here, challenges include trust and liability, as well as regulatory compliance.

While "cheap" often drives the use of plastics, what initial measures can be taken to shift societal norms away from disposable products and embrace more durable choices, considering both regulatory and behavioral aspects?

P. Gabrielli: Subsidies and incentives can be used to soften the burden of businesses and final end-users in using sustainable materials. Moreover, an education process aimed at moving away from consumerism and from the use of the cheapest products will likely be needed to drive our society from the current large and sustained use of plastics.

What steps can be taken to lead the chemical industry towards achieving net-zero emissions, and what role do technology advancements play in this transformation?

P. Gabrielli: The global chemical industry generates USD 4.7 trillion in revenues annually, representing about 4% of global GDP, and directly employs over 15 million people. Overall, the chemical industry strongly relies on capital-intensive and long-lived assets and requires significant investments to change its business models and operations. Any structural changes to be in place by 2050 require action soon, preferably now. In the first phase of the transition, the chemical industry is expected to have access to abundant fossil hydrocarbons as the energy sectors phase out of fossil fuels; in this phase, the CO_2 emissions can be abated through CCS to avoid paying rising CO_2 prices. Then, as fossil fuels are phased out from all sectors, low-carbon feedstocks will be adopted, first through biochemical processes and, eventually, through CCU. However, CCU will need to develop significantly to be able to compete with biomass. All these routes will need to be coupled by increasing recycling rates.

While several leading chemical companies started to set credible climate mitigation targets, creating acceptance and demand for low-carbon industrial products will need multiple diverse actions, including education, changes to industry standards, procurement policies, financial incentives, and low-carbon product standards.

Indeed, while technological advances play a key role in the transition, achieving net-zero emissions will not be entirely under the direct control of the chemical industry. For instance, the impact and effectiveness of circularity and demand-side measures depend on the combination of other societal and technological changes (see above: At the moment only 9% of plastic is recycled, and only about 20% of fertilizers produced from ammonia actually end up in crops that feed the global population). Moreover, the extensive modification and building of plants will require equipment manufacturers, engineering, procurement, and construction industries to expand their capacity significantly. From the perspective of the policy landscape, work to abate the emissions of the chemical industry is being undertaken with an increased sense of urgency worldwide, with commonalities being observed across the globe: (1) low-carbon hydrogen generation (especially via water electrolysis) receives the most attention and policy support among key technologies to enable a net-zero chemical industry; (2) electrification is also receiving significant attention, with low-carbon electricity generation being a top priority for most countries; (3) biomass utilization currently receives the least policy support together with waste processing and circularity, although both are likely to receive additional policy support in the future, moving away from fossil feedstock.

What challenges might arise when trying to implement a circular economy in the chemical industry?

P. Gabrielli: The greatest challenges I see are of an economic, societal, and technological nature. While several production technologies are already available, improved recycling technologies and collection processes are still needed. Furthermore, all net-zero routes will likely be more expensive than the business-as-usual, at least in the first phase of the transition or in the absence of subsidies and incentives that discourage the use of fossil fuels.

From a societal perspective, end-users need to be more involved and engaged in circular economy strategies to ensure the use of sustainable products, which might be more expensive and might require a greater personal effort (for example for collecting and recycling different products). This holds true in the global south, where the consequences of climate change are clear, but the problem might be somewhat less perceived when compared with daily urgent necessities.

How does the availability of resources like land, water, and renewable power sources influence the feasibility of different approaches in different parts of the world? Could you provide examples of regions that are better positioned for this transition and explain the reasons behind their advantage?

P. Gabrielli: In many regions of the world, and for a number of reasons, achieving a net-zero chemical industry without a circular economy and interventions on the demand side will be difficult, or even impossible, due to the availability of land, water, and renewable energy resources.

In most European countries, land resources are limited, and this restricts the production of biomass as a feedstock. In the Middle East and North Africa, scarcity of water makes it difficult to grow biomass or produce hydrogen, which is needed if CO_2 is to replace fossil hydrocarbons as feedstock and serve as a raw material for the chemical industry. The same applies to other large producers like China and India. In China and other big manufacturers of renewable energy technologies, transitioning might be further limited by the water requirements to manufacture technologies that supply carbon-free electricity.

Consequently, transitioning the chemical industry to net zero might entail a restructuring of the international trade in chemicals. Today, with oil and gas as key feedstocks for chemical production, countries with fossil raw materials play a central role. In the future, production might shift to regions with abundant land and water resources, for example in North and South America. In countries like the United States, Canada, Chile, or Brazil, biomass can be grown on arable land for industrial use without endangering the food supply; in addition, water and land resources are available to produce renewable electricity and hydrogen.

Recognizing the absence of a one-size-fits-all solution, why is it crucial to closely examine individual countries and their unique contexts when devising sustainability strategies, and what factors might influence the effectiveness of these strategies?

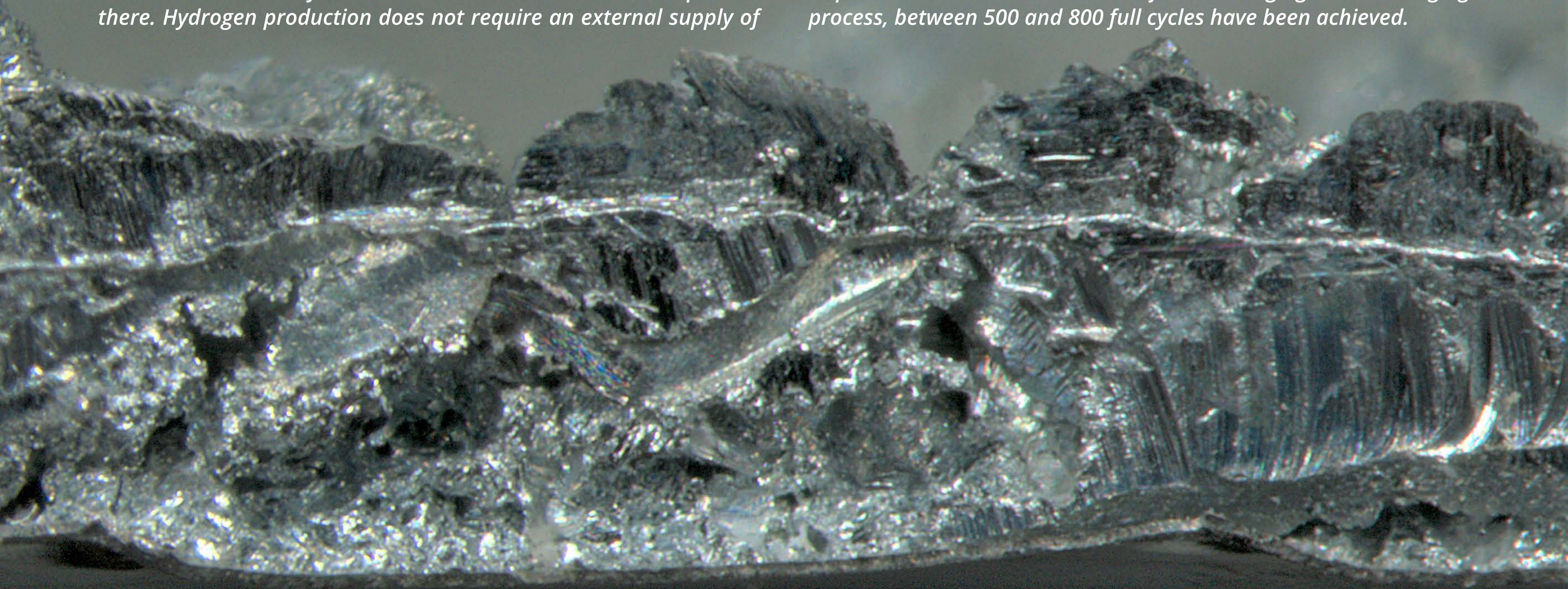
P. Gabrielli: Geographical specificities are crucial when designing a net-zero chemical industry, i.e., when determining the optimal portfolio of solutions based on economic circumstances and local resource availability. Without a local analysis, the technical, environmental, and biophysical viability of a net-zero chemical industry remains less clear for different countries based on local requirements and resources. The latest scientific findings show that a net-zero chemical industry will require integrated solutions that combine net-zero routes with circularity and demand-side measures; such integrated solutions will have to differ regionally based on available resources regarding renewable energy, land, and water availability. Not only this: Further research is needed to perform site-specific assessments, i.e., with a resolution higher than the country level, as biophysical resources can be unevenly distributed within countries (e.g., water basins or regions with available land for biomass growth or renewable generation). High-resolution assessment is key to better quantify the environmental and biophysical impacts of net-zero chemicals on local natural resources and ecosystems as well as the potential for efficiency gains through process integration. In fact, while biophysical resources might be available at a global level, they might be not in the sites where chemicals are currently produced. Such considerations are key to determining the potential reshaping of the chemical industry, both in terms of global trading of chemicals and of re-location of chemical plants, to be moved in places with sufficient availability of local resources.

This interview was conducted by Corinna Herbst, Deputy Editor-in-Chief of GIT Labor-Fachzeitschrift.

A NEW STORAGE CONCEPT with hydrogen production

An electrochemical cell that solves the problem of zinc rechargeability

Zinc-hydrogen storage systems combine the functions of a battery and an electrolyzer in one unit. It can be charged during periods of cheap renewable energy and discharged on demand, delivering both electricity and hydrogen gas. During the charging step, similar to an electrolyzer, oxygen is produced at the gas electrode, but no hydrogen at the counter electrode. Instead, zinc oxide/zincate dissolved in the electrolyte is reduced to metallic zinc and deposited there. Hydrogen production does not require an external supply of energy, but the cell works like a battery, producing electricity and hydrogen simultaneously at low potential. While the primary (only rechargeable) type of this system has been demonstrated previously recharging is now possible thanks to a cycle-stable bi-functional catalyst and a dedicated electronic. One of the most critical issues, the reversible cycling of the zinc electrode, has been solved. Thanks to a special electrical control scheme for the charging and discharging process, between 500 and 800 full cycles have been achieved.



The energy transition in Germany is posing major challenges, especially when it comes to storing green electricity. While renewable energy sources such as wind and solar power are becoming more economical, there is still a lack of cost-effective and efficient storage technologies that can supply the entire country with green energy over an extended period of time. In addition, the intermittent nature of wind and solar power creates intermittent power generation, or "dark clouds," that must currently be offset by the use of conventional power plants. As a result, an energy-intensive dual infrastructure must be maintained, fossil fuels continue to play an important role, and the transition to renewable energy is made more difficult. Cost-effective energy storage is therefore very important, but not yet available. The Zn-H_2 system could play an important role. The material costs are one order of magnitude lower than those of lithium-ion batteries. Although the efficiency is only about half that of batteries, it is more than twice as high as the currently discussed power-to-gas solutions, since there are no significant losses in transporting and storing the hydrogen.

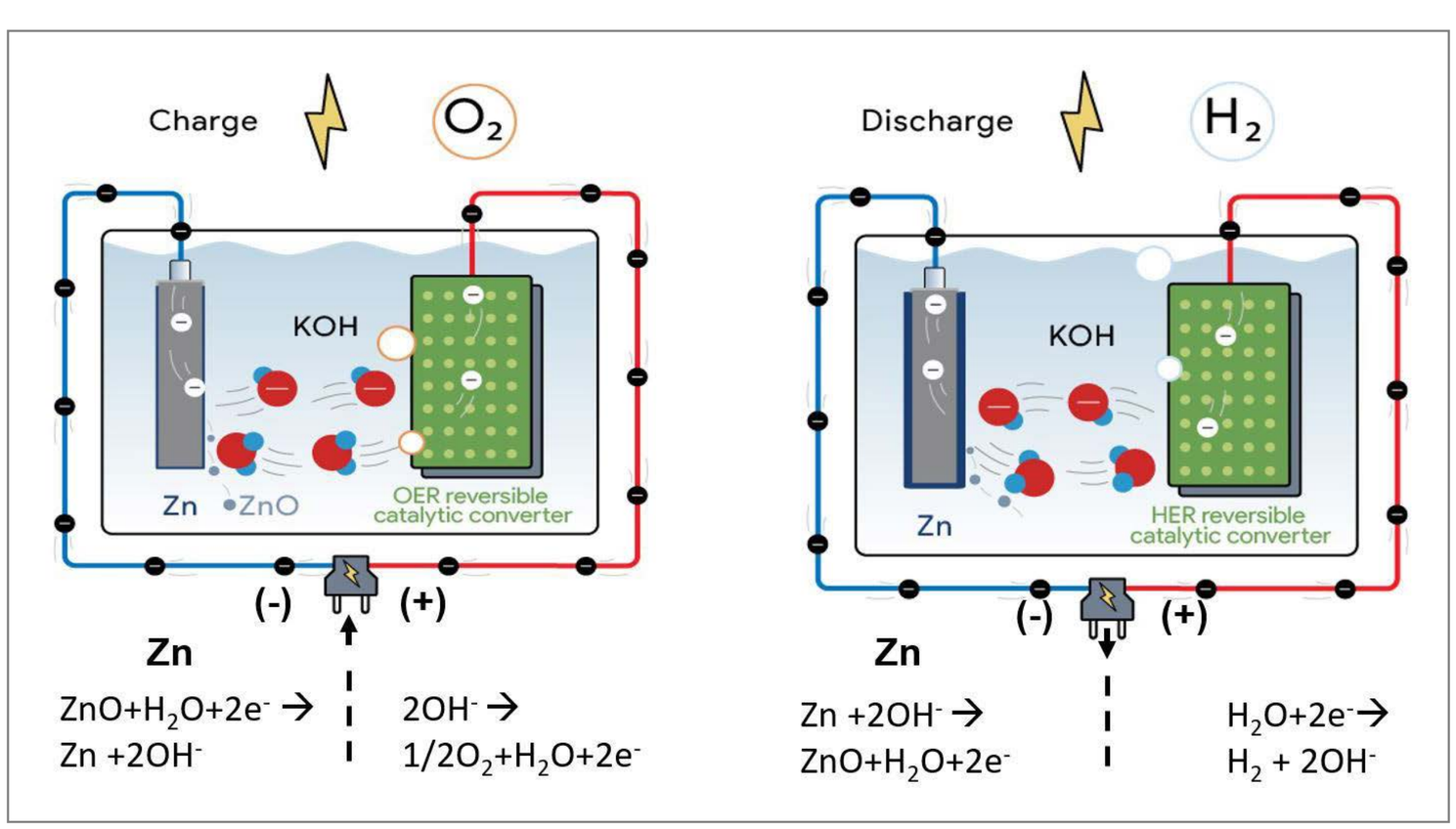


Fig. 1: Functional principle of the Zn-H_2 storage principle and reaction equations.

Dr. Robert Hahn

Robert Hahn received his M.Sc. and Ph.D. degrees in Electrical Engineering from the Technical University of Dresden, Germany, in 1986 and 1990, respectively. He has worked at the Fraunhofer Institute for Reliability and Microintegration (IZM) in Berlin, Germany, since 1994. Dr. Hahn has filed 30 patents in the field of energy systems and has authored or co-authored more than 100 journal and conference publications and book chapters. His research focuses on the development of lithium-ion batteries and novel systems such as aluminum-ion and nickel-zinc batteries, as well as energy storage in electrochemical hydrogen evolution cells.

The operating principle

Figure 1 shows the principle of storage and the reactions at the electrodes during charging and discharging in a simplified form. During the charging process, similar to an electrolyzer, oxygen is generated at the gas electrode while no hydrogen is produced at the counter electrode. Instead, zinc oxide/zincate that is dissolved in the electrolyte, is reduced to metallic zinc and deposited. This process is made feasible by the high hydrogen overpotential of the zinc. During hydrogen production, external energy input is not required. Instead, the cell operates like a battery, concurrently producing electric current and hydrogen at a low potential. As a result, the current density is restricted (5–50 mA/cm^2), being significantly lower than that of electrolyzers. The electrolyte is 30% KOH. The cell has a simple and durable structure that utilizes steel sheets as current collectors and lacks both a separator and gas diffusion electrodes.

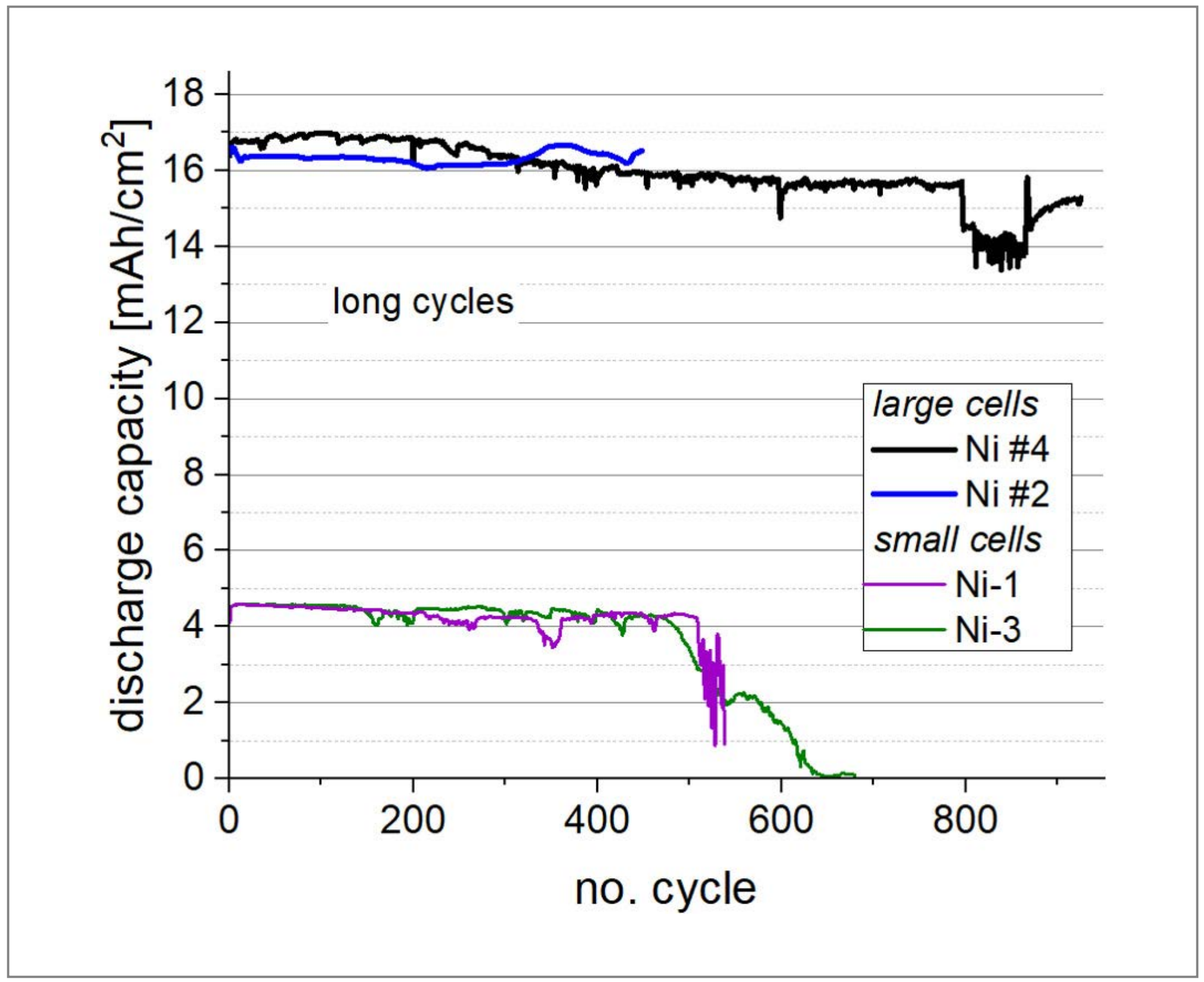


Fig. 2: Long-term cycle tests of various test cells.

Dr. Andreas Schamel

Andreas Schamel has 30 years of experience in automotive engineering across all aspects of powertrain systems. Most recently until the end of 2018 at Ford Motor Co. as Director of Global Powertrain Research and Advanced Engineering. Andreas Schamel received his M.Sc. and Ph.D. degrees in Advanced Automotive Engineering from Loughborough University (UK). Since 2022, he has served as Managing Director of Zn2H2.

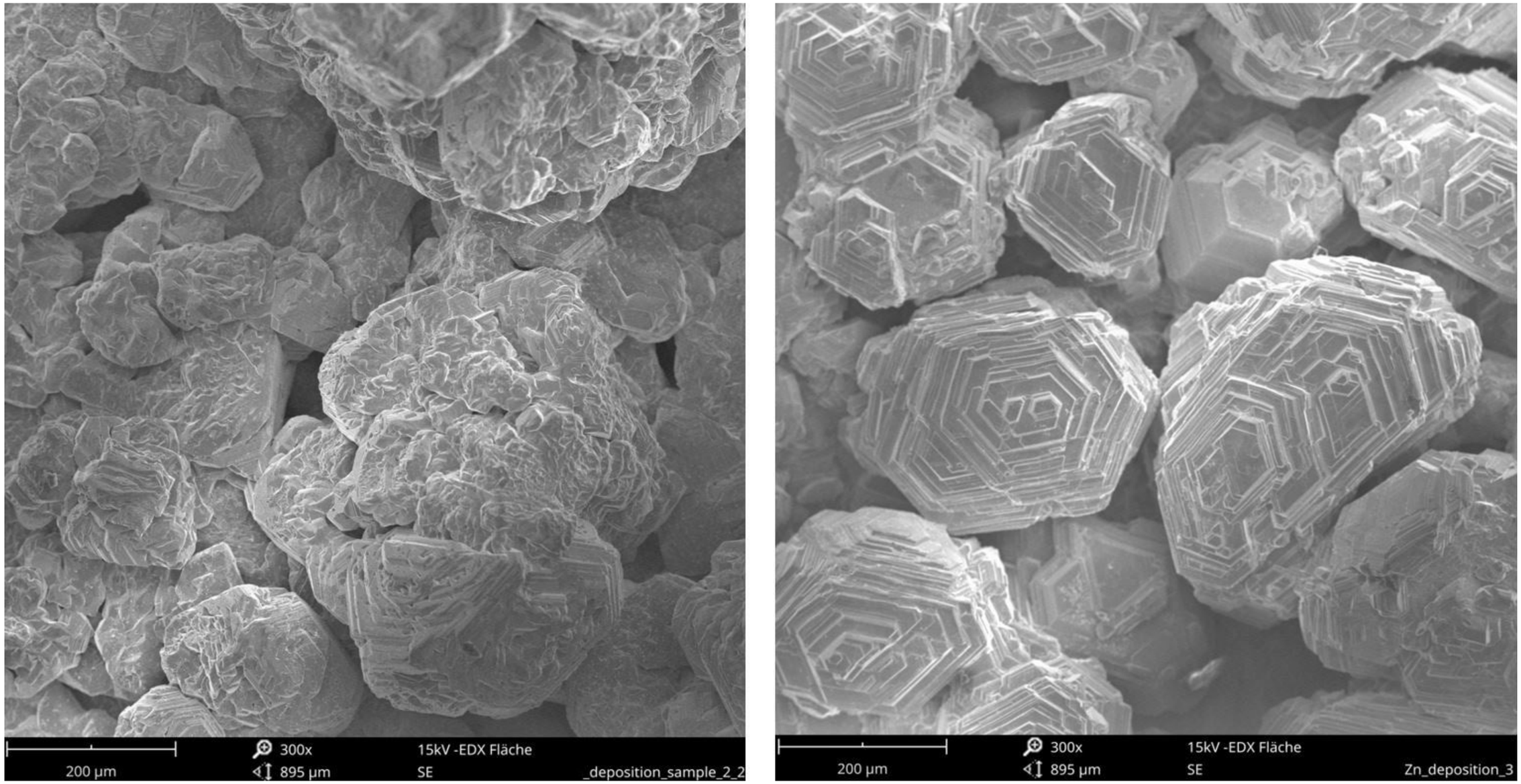


Fig. 3: SEM image of the zinc electrode after charging a capacity of 260 mAh/cm² (left) and 110 mAh/cm² (right).

The gas evolution catalyst electrode

The development of efficient and stable bi-functional catalysts for the hydrogen evolution reaction (HER) and the oxygen evolution reaction (OER) is an active research area in electrocatalysis. Key challenges in developing bi-functional catalysts include achieving high activity and selectivity for both reactions, improving catalyst durability under harsh conditions, and reducing the cost of materials. Bi-functional catalysts perform both hydrogen and oxygen evolution reactions. Using a single catalyst that is active for both reactions can simplify the electrolyzer design and reduce overall costs. Bi-functional catalysts can also boost efficiency by minimizing energy losses from activating separate catalysts at each electrode. During operation in the electrolyzer, catalysts are typically used exclusively for either HER or OER. However, in the Zn-H_2 system, the reaction changes with each charge/discharge cycle resulting in oxidation and reduction reactions of the catalyst electrode.

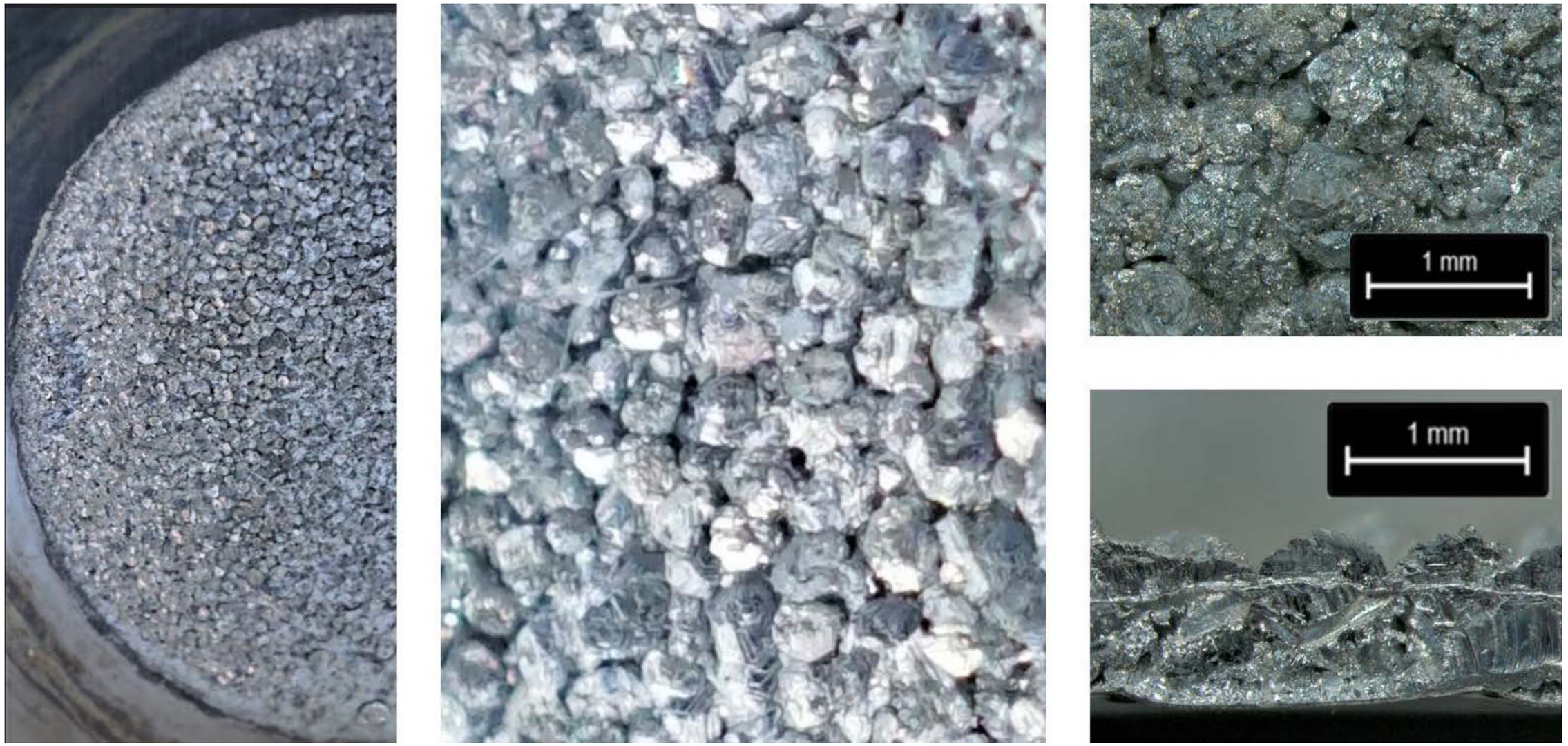


Fig. 4: Light microscope images of the charged zinc electrode 260 mAh/cm². Left: 20 mm diameter section, center: 4x5 mm section, bottom right: fracture cross-section.

Thus, the electrode must be stable over a wide potential range. For the Zn-H_2 system, an electroplated nickel alloy catalyst electrode was utilized. It was shown, that the catalyst withstands more than 500 typical battery cycles and more than 5,000 switching cycles between charge and discharge (Fig. 2, [1]).

The rechargeable zinc electrode

Figure 2 shows that the capacity remains constant during cycling. Unlike secondary batteries, where various aging processes occur that can lead to a reduction in electrode capacity or lithium inventory, there is no mechanism that leads to the loss of zinc. However, it is not to be claimed that the negative electrode is free of problems. On the contrary, the reversible deposition and oxidation of zinc is a problem that has not yet been fully solved, which currently prevents the development of rechargeable zinc-air batteries and limits the number of cycles of nickel-zinc batteries.

Pulse charging techniques are utilized to attain dense zinc coatings at high deposition velocities. This is crucial since voids in zinc on the electrode result in elevated hydrogen production during charging, which is undesirable. It is crucial to prevent both the depletion of zinc ions and internal short circuits during cell charging. Therefore, appropriate electrical charging programs are utilized to guarantee the uniform deposition of zinc. However, electronic control does not only interact during charging but also during discharge. To prevent unfavorable zinc deposition, 100% depth of discharge (DOD) cycles are conducted to fully eliminate all zinc from the electrode. This allows for the deposition to start on a fresh substrate. Figure 2 demonstrates that this process results in slight capacity scattering. However, it is important to note that the end of the cycling is not attributed to the zinc plating or stripping process, but rather to the catalyst electrode's degradation.

Figure 3 displays various images of the charged zinc layer. The thickest layer produced during a single cycle has a capacity of 260 mAh/cm2. This layer exhibits mechanical stability with a shiny, metallic surface, albeit with a coarse-grained, boulder texture.

Efficiency

The electricity storage efficiency of the Zn-H_2 system in combination with a fuel cell can be characterized by dividing the discharge voltage by the charge voltage, defining the round-trip efficiency. Neglecting hydrogen losses is possible as hydrogen is produced upon request. Long-term self-discharge can be circumvented by draining the electrolyte from the cells. In this scenario, the charge and discharge voltages are 2 V and 1 V, respectively, assuming a fuel cell voltage of 0.7 V. This gives an efficiency of 50%. The overvoltage of the zinc deposition and dissolution ranges from 10 to 30 mV and can be disregarded compared to the OER and HER overvoltages. If the system is utilized for hydrogen production instead of electricity generation, the efficiency is over 80%.

Conclusion

A new storage principle, Zn-H_2, was assessed via single cells and presents favorable parameters for cost and long-term stability, making it a promising option for various applications. The initial applications could include emergency power supplies, solar storage, and seasonal energy storage; however, significant technical challenges, including up-scaling and system integration, need to be addressed. Adaptation of existing solutions, such as KOH-tight housings and the separation of liquid and gas in electrolyzers, may be necessary. Furthermore, research is being conducted on fundamental studies, such as examining the impact of zinc on the catalyst and implementing measures to enhance power density.

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Fuels from sunlight and air

A pioneer technological demonstration leads the path toward sustainable aviation

A research group at ETH Zurich has developed a thermochemical process technology that can produce carbon-neutral transportation fuels from concentrated sunlight and ambient air. Here, Dr. Cecilia Kruszynski, editor of Wiley Analytical Science, interviews Prof. Aldo Steinfeld, who directed this multi-year research effort that can shape the fuel refineries of the future and especially contribute to the long-term decarbonization of the aviation sector.

Fig. 1: Photograph of the solar mini-refinery at ETH Zurich for the production of drop-in fuels from sunlight and air. The process chain integrates three thermochemical conversion processes. Firstly, the extraction of CO_2 and H_2O directly from atmospheric air. Secondly, the solar thermochemical splitting of CO_2 and H_2O to produce syngas. Thirdly, the processing of syngas into hydrocarbon fuels [1].

Prof. Aldo Steinfeld

Aldo Steinfeld holds the Chair of Renewable Energy Carriers at ETH Zurich. His fundamental research focuses on heat/mass transport phenomena, multi-phase reacting flows, thermochemistry, and functional redox materials. These are applied in the development of technologies for solar power and fuel production, direct air capture, energy storage, and carbon-neutral sustainable energy systems.

You have had an impressive career at ETH Zurich and recognition through various awards. Could you share some key moments or experiences that have shaped your journey in solar energy research?

A. Steinfeld: As I approach retirement, it is a good time to reflect on past experiences. It has been an amazing journey, filled with many joyful moments but also with plenty of failures, which are inherent in pioneer research. One of these special moments in my career was witnessing, together with my team, the kickoff operation of our solar mini-refinery mounted on the roof of the Machine Laboratory Building and observing the first drops of methanol produced from nothing other than sunlight and air. This setup represents the culmination of a decade-long R&D in several fundamental topics that were essential for the success of the project, including developing redox materials and structures, analyzing the thermodynamics and kinetics, modeling heat and mass transport at high temperatures, designing high-flux optics, and last but not least engineering the solar reactor for efficiently producing solar fuels. As for the many failures we encountered along the way, we overcame them by applying good engineering and perseverance.

You're known for your work in solar fuels. Can you provide an overview of solar fuels' significance and potential applications in sustainable energy production?

A. Steinfeld: Since the early days of my career, the signature portion of my research has been the production of solar fuels. Solar energy is clean and unlimited, but solar radiation reaching the earth is diluted, intermittent, and unequally distributed. Long-term storage and long-range transport of solar energy are essential for a transition away from fossil energy. If we concentrate the diluted sunlight with the help of parabolic mirrors and then capture that radiative energy with the help of solar receivers, we would be able to obtain heat at high temperatures for driving a thermochemical transformation and producing a storable and transportable fuel. This thermochemical pathway to solar fuels uses the entire solar spectrum and offers potentially high production rates and efficiencies. Initially, our focus was on the production of hydrogen from water. Later, we re-directed our focus to target drop-in fuels from water and CO_2. Drop-in fuels are synthetic alternatives for petroleum-derived liquid hydrocarbons such as gasoline and kerosene, which are fully compatible with the existing global infrastructures for storage, distribution, and end-use of transportation fuels. Especially kerosene is indispensable as a jet fuel for long-haul aviation because of its high specific gravimetric energy density. In our solar refinery, we can produce drop-in fuels from sunlight and air [1].

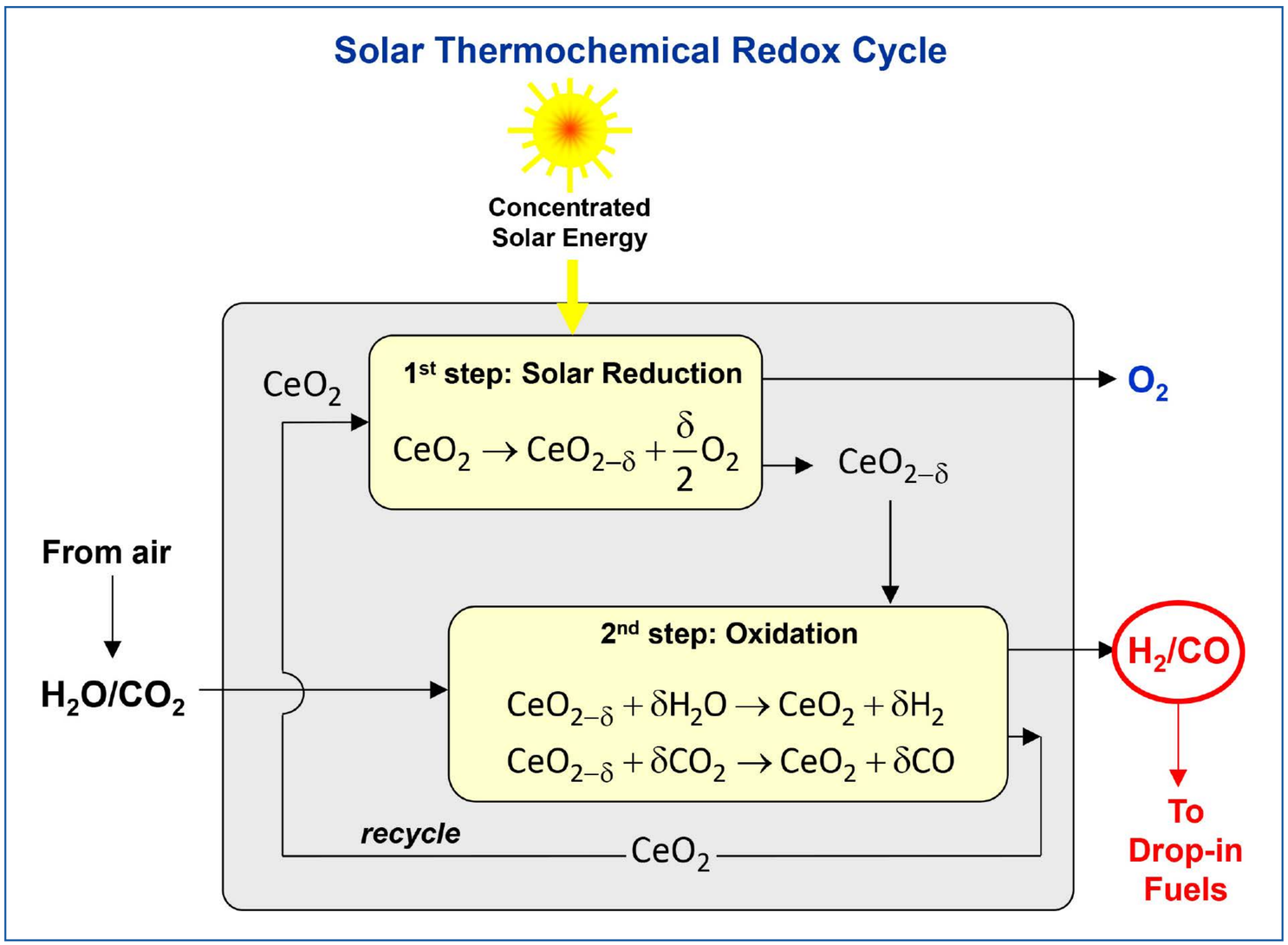

Fig. 2: Schematic of the 2-step thermochemical cycle for splitting CO_2/H_2O into separate streams of CO/H_2 and O_2 via reduction-oxidation (redox) reactions using non-stoichiometric ceria (CeO_2). In the first endothermic step, ceria is thermally reduced to generate O_2 using concentrated solar process heat. In the second exothermic step, the reduced ceria is re-oxidized with CO_2 and H_2O to generate CO and H_2 (syngas). δ denotes the nonstoichiometry – the measure of the redox extent and thereby the fuel yield per cycle.

How does this solar refinery function?

A. Steinfeld: Our solar refinery (Fig. 1) serially integrates three thermochemical conversion units. First, the direct air capture unit which co-extracts CO_2 and H_2O from ambient air via an absorption-desorption process using an amine-functionalized sorbent. Second, the solar redox unit converts CO_2 and H_2O into a tailored mixture of CO and H_2, so-called syngas, via a thermochemical redox cycle. And third, the gas-to-liquid unit which converts syngas to methanol or hydrocarbon fuels, such as kerosene.

Is this synthetic kerosene carbon neutral?

A. Steinfeld: Yes, it is truly a carbon-neutral fuel because solar energy is used for its production and because it releases only as much CO_2 during its combustion as was previously extracted from the air for its production. The solar fuel production chain's life-cycle assessment indicates 80% avoidance of greenhouse gas emissions with respect to fossil jet fuel and approaching zero emissions when construction materials, e.g., steel and glass, are manufactured using renewable energy [2].

One of your notable contributions is the development of the ceria-based thermochemical redox cycle for converting CO_2 and H_2O to syngas using solar energy. Can you explain how this process works?

A. Steinfeld: This thermochemical redox cycle (Fig. 2) is at the heart of the solar refinery. It comprises the endothermic reduction of ceria, followed by the exothermic oxidation of the reduced ceria with CO_2 and H_2O to generate a tailored mixture of CO and H_2. Ceria is thus not consumed and the net overall reactions are the co-splitting of CO_2 and H_2O:CO_2 = $CO+½O_2$ and H_2O = $H_2+½O_2$, but with the fuel and O_2 being generated in separate steps and thus avoiding the formation of explosive mixtures and obviating the need for high-temperature gas separation. The cycle is driven by concentrated solar energy.

Animation of the solar refinery for the production of drop-in fuels from sunlight and air.

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In your recent publication [3], you discuss the challenge of isotropic topology in ceria structures. Could you elaborate on the significance of hierarchically ordered structures and how they improve the efficiency of solar-driven fuel production?

A. Steinfeld: The solar reactor for effecting the thermochemical redox cycle consists of a cavity-receiver containing a porous ceramic structure made of the redox material ceria. This structure is directly exposed to concentrated sunlight and reaches temperatures of up to 1,500 °C required for the reduction step of the cycle. With this arrangement, the ceria structure serves the functions of both radiative absorber and redox material. Until now, structures with isotropic porosity have been applied, but these cause an exponential attenuation of the incident radiation, which leads to an undesired temperature gradient along the radiation path, detrimentally affecting the solar reactor performance. In contrast, hierarchically ordered topologies with stepwise optical thickness enable the volumetric absorption of concentrated solar radiation. This, in turn, ensures that the entire volume of the porous structure reaches the reaction temperature and contributes to fuel generation.

How does the use of direct ink writing (DIW) technology play a role in creating hierarchically channeled structures for ceria and optimizing their radiative absorption properties?

A. Steinfeld: We applied a DIW-based 3D printing process using a novel ink formulation to manufacture robust and stable ceria structures with graded topologies.

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Fig. 3: Photograph of the solar tower fuel plant located at IMDEA-Energy, Madrid. A field of sun-tracking heliostats focuses the direct solar irradiation onto the solar reactor positioned at the top of the tower. The solar reactor co-splits CO_2 and H_2O via the ceria-based thermochemical redox cycle and produces a desired mixture of CO and H_2 (syngas), which is finally processed via Fischer-Tropsch synthesis to kerosene [4].

We further analyzed the complex interplay between radiative transfer and thermochemical reaction by performing redox cycles in a thermogravimeter under high-flux radiation. We showed experimentally that hierarchically channeled structures achieved a higher and more uniform temperature profile compared to that of state-of-art isotropic structures, doubling the specific fuel yield for the same solar flux input equivalent to 1,000 suns [3].

Two spin-off companies emerged from your laboratory, Climeworks and Synhelion. Can you tell us more about the creation of these two spin-offs and the technologies they are commercializing in the context of carbon capture and solar fuel production?

A. Steinfeld: My former doctoral students founded Climeworks and Synhelion based on the science and technologies developed in my lab. Climeworks commercializes the technology for direct air capture. Synhelion commercializes the technology for the production of solar fuels. I believe both companies will make significant contributions to combating climate change by removing CO_2 from the atmosphere and by implementing carbon-neutral drop-in fuels in the transportation sector.

The production of carbon-neutral transportation fuels and, in particular, the so-called sustainable aviation fuels (SAF), has become a global challenge. What do you see as the most significant hurdles and opportunities in producing SAFs using solar energy on a large scale?

A. Steinfeld: Increasing solar-to-fuel energy efficiency is essential for improving the economic viability of solar-made SAFs, and my group and many other research groups around the world are investing major efforts in this direction by discovering superior redox materials and designing more efficient solar reactors. Furthermore, scaling up the solar reactor is critical for advancing technological readiness and we have demonstrated the production of solar kerosene from H_2O and CO_2 in a solar tower (Fig. 3) [4]. However, given the high initial investment cost for building the first generation of industrial-scale solar refineries, policy support is additionally required to see widespread deployment, leading to concomitant cost reductions initially through scaling effects and process optimization, and then through mass production of key components and learning-by-doing. I believe the policy instrument most suited to bring solar fuels to market would be a quota system mandating airlines to have a minimum share of SAFs in their total jet fuel volume. This quota would be initially small and rise each year, leading to new solar refineries, and that in turn to falling costs, just as we observed with solar power. For example, the EU already adopted a plan to impose a quota of 2% SAF in 2025, rising to 20% in 2035, and gradually to 70% in 2050.

Would it be feasible to have a 100% quota met by solar-made SAFs?

A. Steinfeld: In principle, yes. Global jet fuel demand can be met by solar-made SAFs by utilizing less than 1% of the worldwide arid land, which does not compete with food production. As demonstrated, the ingredients needed are sunlight and air.

Finally, what are your aspirations for the impact your research will have on the world's transition to sustainable energy solutions?

A. Steinfeld: I very much hope that, in the not-too-distant future, we can fly with solar kerosene!

This interview was conducted by Dr. Cecilia Kruszynski, editor of Wiley Analytical Science.

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ChatGPT For Dummies:
Navigating AI and unleashing creativity with confidence

ChatGPT For Dummies provides a fun and easy overview of the essentials of applying this evolving artificial intelligence platform to your work. This book breaks down how to use the technology responsibly and adapt it for best use, while also addressing serious concerns about the potential negative impact of this program.

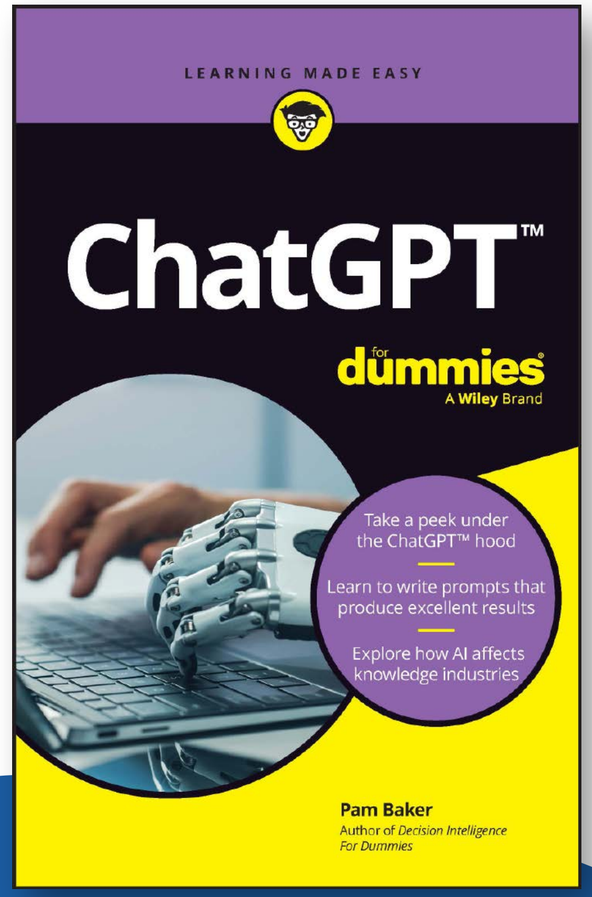
Written in the friendly and accessible *Dummies* style, expert author, Pam Baker, demystifies artificial intelligence by showing readers how this technology can benefit both professional and education fields and can change the world. Made for professionals whose careers might be impacted by ChatGPT as well as those simply interested in learning about this new technology, ChatGPT For Dummies guides readers through this new technology trend.

The first part of this book shows readers the beginning steps of using ChatGPT, including setting up an account, understanding the program, and discovering the differences between other GPT and AI products. Readers gain insight into what's different about ChatGPT but also come away understanding its limitations. Despite ChatGPT being exposed to massive training datasets, it's still a flawed program. It may produce wrong or offensive answers, which is why it's important to fact-check its responses and create specific and detailed prompts.

Throughout the book, Baker educates readers on both the uses and potential problems of using ChatGPT:

- Incorporation and benefits of ChatGPT in business software
- Uses of AI in the classroom for both educators and students
- Change of virtual assistants to knowledge assistants
- Dangers from lack of security or privacy shields, plagiarism, copyright, and spread of misinformation.

This article was written by Hanna Sytsma.



Pam Baker is a freelance journalist and industry analyst focused on emerging tech topics like artificial intelligence, quantum computing, swarm computing, metaverse, data analytics, edge computing, and others. She is the author of Decision Intelligence For Dummies and Data Divination: Big Data Strategies.

AGAMEDE: Bridging ancient wisdom and cutting-edge technology

High-throughput screening system for AI-driven experimentation

Agamede is the first European scientist in history. Homer wrote in the 12th century BC that she knew the healing power of all herbs and mixed them properly. High-throughput Screening System (HTS) developed at the Institute of Bioorganic Chemistry Polish Academy of Sciences (ICHB PAS) with the Labomatica, Mitsubishi Electric, and Perlan Technologies partnership was named in her honor. The uniqueness of AGAMEDE is not related to the automation of laboratory work. It relies on the integration of automation and artificial intelligence using Labomatica's Gene Game software. This combination makes the system a 'closed loop'. The robotic devices prepare the experiments, read the results at a set time, and interpret the data to prepare the next experimental cycle. The operator's tasks are reduced to formulating the research problem and designing the experimental model to test it. The tasks of the system are to perform the experiments 24 h a day and deliver the solution. Here, the editor interviews Dr. Radosław Pilarski, Head of Laboratory Automation and Robotic Group at IBCH PAS, about this innovative approach.

How did the idea for AGAMEDE come about? Could you share the history and background of the project?

R. Pilarski: I think the origins should be traced back 20 years when I was working on my Ph.D. on the pharmacological properties of the Amazonian liana, cat's claw (Uncaria tomentosa) under the supervision of Professor Krzysztof Gulewicz. It came to the attention of ethnopharmacologists in the 1970s, who were studying the medicinal system of the Asháninka tribes of the Peruvian Amazon. The publicity it quickly gained worldwide for its anticancer properties led to the mass export of its bark and the destruction of natural populations. At the time, I thought I would develop an in vitro cell culture system of this plant and begin to isolate the secondary metabolites. This is where the problem arose because the standard Murashige & Skoog medium for plant cell cultures did not work and I had to prepare my own formulation for this plant. This is how I became interested in John Holland and David Goldberg's papers on genetic algorithms, which was an effective AI method for finding the composition of the medium from many possible combinations of mineral salts, phytohormones, and vitamins. It quickly became apparent that I could not manually prepare the number of formulations required by the algorithms, so my thinking followed laboratory automation. Unfortunately, only thinking, because as a Ph.D. student, I did not have the funds to purchase even the simplest pipetting station.

What happened next?

R. Pilarski: The idea of automatic optimization of bioprocesses using genetic algorithms has been permanently set in my mind but the expense of automation was long out of my reach. In 2013, I was awarded a grant to optimize U. tomentosa media, which enabled me to purchase an Agilent Bravo pipetting station. A breakthrough 3 years later, when I was invited to participate in the EPICELL project by Professor Wojciech T. Markiewicz. This was a strategic medical project in Poland aimed at developing a method for treating post-infarction patients using pluripotent cells. My task was to lead a team of more than 20 people building a laboratory automation system to develop media for the transformation of myoblasts taken from the patient into cardiomyocytes. We then actually started work on AGAMEDE, which we are still expanding today.

Can you provide a technical overview of the system? How did the design and engineering work proceed?

R. Pilarski: When starting design work on the system, we theoretically had three approaches to choose from. We could have purchased a large pipetting station and integrated it with peripheral instruments, built a glass robotic cell with a small six-axis robot to operate the instruments, or built a laboratory and filled it with robots and automation. We didn't think long and chose the most ambitious for an automated laboratory.

Dr. Radosław Pilarski

Scientist, designer, roboticist, and entrepreneur. Head of Laboratory Automation Group at Institute of Bioorganic Chemistry Polish Academy of Sciences. President of the Polish Laboratory Automation Society (PLAS). Chief Executive Officer of Labomatica Ltd – a biotech company involved in lab automation, instrument construction, and AI systems for bioprocess optimization. Editor-in-Chief of the Polish edition of the 23-volume Great Medical Encyclopaedia (UTET SPA Planeta DeAgostini SA). His scientific interests include evolutionary biology, biotechnology, ethnopharmacology, personalized medicine, game theory, artificial intelligence, and robotics.

We definitely went for it without compromising. No one realized the big risk due to lack of experience and limited resources. We divided the room into two parts: robotic and operator. In the robotic section, we placed a large Mitsubishi Electric RV-7FLM industrial robot to operate the devices, which we set up in a semicircle on dedicated tables. To increase throughput, we also added a second Mitsubishi Cobot Assista robot on a 4-m running track. We lined the walls with ISO-1 grade cleanroom steel panels, raised the floor by 25 cm, and poured resin. This gave us the space to run more than 1,500 m of power, signal, and pneumatic cables. In the operator area, we placed tables with computer terminals to operate the system. Here, we draw in air, which we cool or heat with an exchanger in the ventilation line and introduce into the robot room through ceiling-mounted HEPA filters, maintaining a constant temperature and an overpressure of about 20 Pa. The resulting cleanliness of the robotic room is similar to that of a laminar hood and allows for research using cellular models. We used glass panels to divide up the room, which gave us excellent visibility of the robots and equipment. Additionally, we hung 4K monitors and installed 10 cameras in different locations and with different lens focal lengths.

Which scientific instruments are included in the AGAMEDE system? What guided you in their selection?

R. Pilarski: We managed to integrate more than 30 devices, not counting sensors, converters, relays and other actuators, and control modules. Our guiding principle was to build a laboratory where robots could replicate molecular biologists working on cellular models. The system supports microplates in SBS/ANSI format with different numbers of wells provided for in this standard, but we mostly use 96- and 384-well plates. We have a variety of devices, including single- and multi-position plate stackers Agilent VStack, Agilent BenchCel, and Thermo Cytomat. We use labelers and barcode readers at various points in the system to label samples and track them. We store the chemical library in the Labomatica BioInsert 360 biobank and transfer it to assay plates with an Echo Beckman Coulter acoustic dispenser. This is an amazing device that allows the transfer of up to 2.5 nL of liquid. We measure fluorescence, absorbance, and luminescence with the BMG Clariostar and Biotek Cytation plate readers. And microscopic imaging and high-content analysis are provided by Perkin Elmer's Opera Phenix system, which generates huge amounts of data and is to biologists what the Hubble space telescope is to astronomers. In addition, we have many other sample processing equipment such as pipetting stations, CO_2 incubators, plant chambers, piercers, centrifuges, (de)sealers, and (de)cappers.

Quite a serious fleet of machines but how do you control the operation of so many devices and the course of the experiments?

R. Pilarski: We use various IT systems implemented on high-speed servers with large disk capacities. We use Agilent VWorks Scheduling Software with our plugins to control the robots and automatics. We also have dedicated LIMS software from Labomatica with modules for biobank management, plant control, instrument monitoring, electronic laboratory notes, and remote control.

Remote control? Can you operate the lab at home?

R. Pilarski: Yes, and we often do, because AGAMEDE is a 'smart lab' system. We have integrated solutions typical of smart homes. Except that instead of turning the garage door on and off or the heater on the way home, we turn appliances on and off, turn lights on or off, manage camera images, and, above all, control robots and conduct remote experiments.

Could you explain the concept of the 'closed loop' setup in the robotic system? How does it contribute to its autonomous operation?

R. Pilarski: This is a concept introduced by the futurologists of the 20th century, which has been considered under the various names of ‘Intelligent Laboratory Automation’, ‘Total Laboratory Automation,’ or ‘Automatic Drug Factory’. In the traditional process, automated assistance is linear, meaning that after the experiment is over, human input is required to analyze the data and plan the next step to verify the research hypothesis. For example, feeding samples into a chromatograph using an autosampler was introduced more than 50 years ago, which was a breakthrough as it freed up tedious laboratory work even though interpretation of the spectra had to be handled by a human. In closed-loop systems, laboratory automation is coupled with an artificial intelligence module that eliminates the operator from subjectively planning the next experiments or interpreting the results. The role of the operator is to define the problem and experimental model, the role of the automation is to perform the experiments and provide the solution or answer.

How does AGAMEDE utilize artificial intelligence (AI) to interpret experimental data? What role does Labomatica's Gene Game software play in this process?

R. Pilarski: AGAMEDE does not interpret the data in the sense of pointing out reaction mechanisms, discussing results, or rational explanations. It uses genetic algorithms and neural networks to iteratively guide experiments in the desired direction. Imagine trying to optimize a cocktail of cytostatic drugs for personalized cancer therapy.

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We have isolated and multiplied cells from a patient and are trying to propose the most optimal personalized therapy using 10 different compounds. We want to synergize the best effect by lowering their molar concentrations and thus minimize side effects. In other words, we need to mix the chemical compounds in the right way, but we don't know how to do it and we need to test all possible combinations. If we test 10 concentrations of each compound, the number of combinations required to be tested would be 10 billion (10^{10}). Obviously, we cannot perform that many experiments even in the most efficient HTS system, but we can test 10,000 different formulations in small volumes by placing them on microplates, assess the viability of the cells after their administration, and prepare another experiment with the same number of samples based on the results. In short, it boils down to eliminating unsuccessful formulations and creating variations of the most promising ones. By repeating the tested 10,000 formulations in cycles, we optimize the cocktail and get closer to our target. Labomatica's Gene Game software guides this process [1], alternating between creating and testing different formulations in an appropriate manner, and controlling the laboratory automation. This is the heart of the AGAMEDE system, which mimics biological evolution in silico. From a Darwinian point of view, a formulation in a well is an individual, a microplate is a population, an iteration is a generation, and the stated research goal is natural selection.

What are the potential applications of the robotic system? How can it be utilized in various fields?

R. Pilarski: The AGAMEDE system was built for drug discovery, combinatorial screening, and bioprocess optimization. It can also be used for diagnostics and in theoretical research in life sciences. For example, it will be screening a chemical library to find an inhibitor of a specific metabolic pathway, optimizing media for stem cells to differentiate into different cell types, formulating chemical solutions for efficient crystallization of biological proteins, or determining optimal buffer compositions to enhance the efficiency of enzymatic reactions. The system makes it possible, for example, to determine the tertiary structures of macromolecules such as proteins or to model evolutionary processes to better understand and verify theories, concepts, and hypotheses.

What is the capacity of the system?

R. Pilarski: The capacity of the system depends on the type of biological assays and used models. Some of them are simple and fast, others are complex and require time for either cell incubation or chemical reaction. It can be assumed that for simple biochemical models, AGAMEDE allows analysis of more than 1,000,000 samples per week and efficient searching of a multi-dimensional data space formed from a quadrillion possible solutions (1024). An integral part of the system is the EU-OPENSCREEN chemical library of more than 115,000 compounds with different biological properties used for drug and active substance searches [2]. Of course, the system can operate 24 h a day, 7 days a week.

Looking at AGAMEDE, one cannot help but notice the futuristic design for which you have won many international design awards, including the prestigious iF Design Award. Finally, can you say what guided you in adopting the rare combination of artistry and cutting-edge technology?

R. Pilarski: I am convinced that the most interesting ideas arise at the intersection of different disciplines. At AGAMEDE, we are active in the fields of science, engineering, and design. We try to take each one very seriously. In design, we wanted to present a continuum between antiquity and futurism. The visual identity draws on ancient Greece. It pays tribute to the origins of our civilization's scientific thought and, above all, to women in science. On the poster depicting the visualization of the mythical AGAMEDE, we added futuristic elements. This created the figure of an ancient sculpture and a cyborg. The blue, illuminated brain, and the bit motif are a combination of human thought processes and artificial intelligence. The figure is meant to be associated with a humanoid robot that solves combinatorial tasks symbolized by folding cubes like in a Rubik's cube. In designing the robotic and operator space, we broke with previous standards. The clean room for the aseptic cell culture procedure was given a new face thanks to large, sealed windows and panes of glass. The lighting of the apparatus by stage lights added a modern touch. Three beams of light in blue, red, and white mix on the AGAMEDE apparatus and the snow-white Corian tables. We wanted AGAMEDE to be like a Lamborghini, with a beautiful appearance and an engine offering excellent performance.

This interview was conducted by
Dr. Cecilia Kruszynski,
editor of Wiley Analytical Science.

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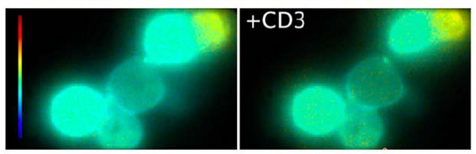
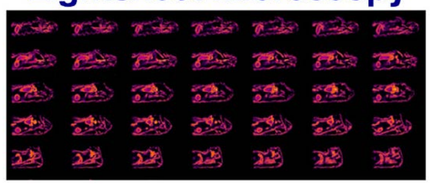
Time-resolved wide-field imaging

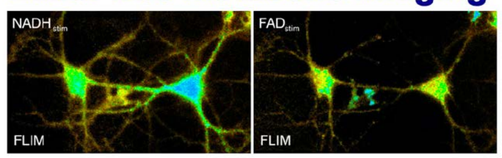
FLIM/FRET of biosensors



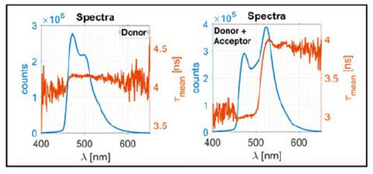
Lightsheet microscopy



Label free metabolic imaging

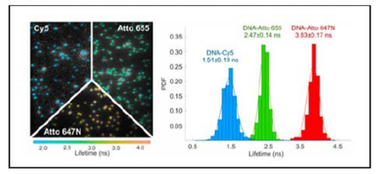


Time resolved spectroscopy

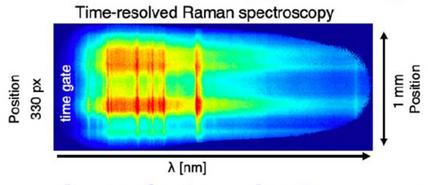


LINCam

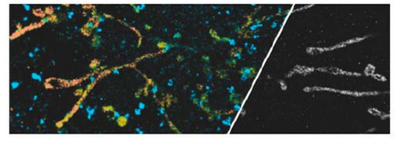
Single molecule detection



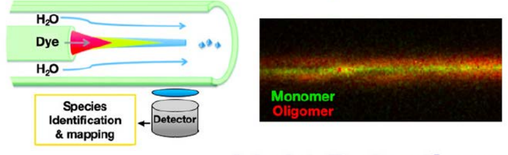
Raman spectroscopy & imaging



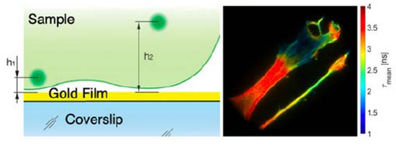
Superresolution microscopy



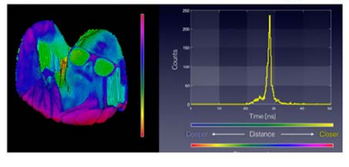
Dynamics of chemical processes



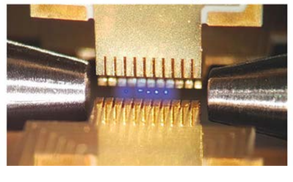
Metal induced energy transfer (MIET)



Light Detection and Ranging (LIDAR)



Quantum optics



Spinning disc confocal microscopy

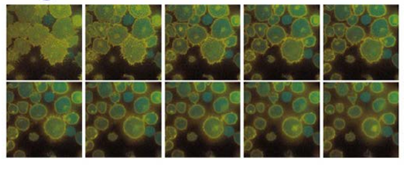


Fig. 1: Applications of the LINCam.

We report on an easy-to-use single-photon counting system for time-resolved, ultra-sensitive imaging from the ultra-violet to the near-infrared spectral range. In contrast to conventional charge-couple devices (CCD) or complementary metal oxide semiconductor (CMOS) based cameras, it utilizes a patented, pixel-free quantum sensor consisting of a photocathode, multi-channel plates for image intensification, and a resistive anode with electrically isolated fields for positional sensitive read-out, enabling 40 ps of timing accuracy combined with 1,000 x 1,000 distinguishable spatial bins. The dark count rate over the entire detector area (17 mm diameter) is below 50 photons per second, i.e., the sensor works almost noise-free with up to 1 MHz single-photon count rate. This extraordinary signal-to-noise ratio of the camera system enables high image quality under extreme low light conditions. As the acquisition system is operated in the single-photon-counting regime, the image data is not stored in a frame-based format but in an event-based manner, i.e., a table of all captured information of the detected photon, like positional coordinates, relative timing to a sync-signal, absolute arrival time and up to ten bits of additional information from external triggers. With this information, arbitrary frames and movies, based on various conditions can be reconstructed from the photon-event database. In combination with a pulsed light source, our imaging system transforms any fluorescence wide-field microscope into a powerful instrument for measuring fluorescence lifetimes. This enables total internal reflection fluorescence (TIRF), light-sheet, or spinning disk method to be used for fluorescence-lifetime imaging microscopy (FLIM) acquisition. The main applications are single-molecule imaging, high-resolution microscopy, label-free metabolic monitoring of NADH and FAD, time-resolved spectroscopy, and quantum optics. In this article, we describe a few selected examples of the applications mentioned above.

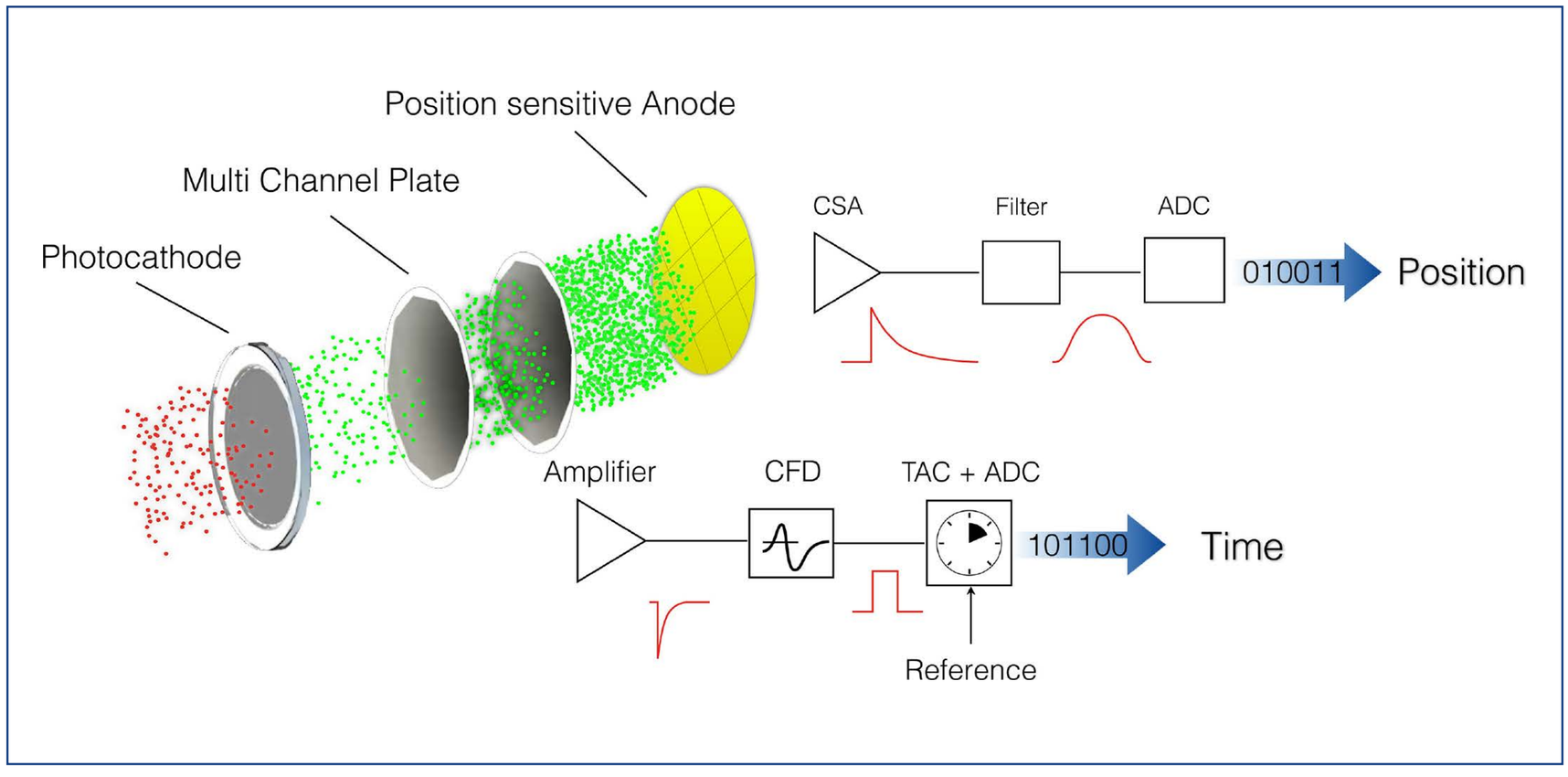


Fig. 2: Operational Principle: The sensor of LINCam consists of a photocathode, a stack of multi-channel plates and a resistive anode with isolated electric fields. Photocathode: It transforms photons into photo-electrons; Multi-Channel Plate (MCP): Acts as electron amplifier, one MCP has the amplification potential of 10,000 per input electron, a chevron assembly achieves 107 per input electron. The resistive anode with isolated fields is used to determine the position of the incident photon at the photocathode. The detector head generates two continuous data streams: One for position, one for timing. CSA: Charge Sensitive Amplifier, ADC: Analog Digital Converter, CFD: Constant Fraction Discriminator, TAC: Time to Amplitude Converter.

Video 1: Tutorial on the operational principle of LINCam.

Introduction

Besides spatial and temporal resolution, sensitivity is the main challenge in functional microscopy of living cells. Too little attention is often paid to both the sensitivity of the detection system and the light dose used for illumination of the sample, although these factors can significantly influence the results and, in the worst case, falsify them or render them worthless. For this reason, we have developed a low-light, wide-field fluorescence lifetime imaging method that does not (or minimally) affect the long-term viability of cells or tissues [1,2]. Low-light FLIM is based on a highly light-sensitive photomultiplier detector that works at intensities below 5 mW/cm^2. The method can be used for many applications in life sciences [3– 12] and quantum optics (Fig. 1) [13–15]. It enables precise measurements of complex fluorescence decays of labeled or native, label-free samples, even under UV light. Here, we present some selected applications of the method in addition to a brief summary of the functional principle (Fig. 2) [16,17].

Operational principle

The low-light detection method described here is based on an extremely sensitive single-photon counting wide-field detector system (LINCam, Photonscore GmbH, Magdeburg, Germany). The core of the camera comprises a vacuum-sealed position-sensitive photomultiplier tube (PMT) with a high quantum efficiency photocathode, a stack of micro-channel plates (MCPs) acting as electron multiplier by secondary emission of electrons, and a resistive area anode with numerous isolated fields from which the space coordinates of the incoming photon is calculated by an artificial neuronal net (Figs. 2, 3) [16, 17].

Consequently, the wide-field quantum detector operates without a pixel-based sensor element and provides spatial information with a diffraction limited positional accuracy for each detected individual event. The spatial resolution is comparable to a conventional camera with approximately 1,000 x 1,000 pixels, but in contrast to pixel-based sensors (e.g., CCD, CMOS, SPAD arrays) the data acquisition works at an extremely low noise level. Due to the photon-counting mode operation principle, the event detection is Poisson-limited, hence, the total dark count rate of the whole sensor area is below 50 counts per second during data acquisition.

The detector operates in single-photon counting mode and allows acquisition of timing information with temporal resolution

below 40 ps (FWHM) for incoming events concurrent with position. The exceptional timing performance of MCPs enables the acquisition of fluorescence decay information with the highest precision. In contrast to traditional detector systems, which save the captured information in the form of images, the LINCam saves all parameters in a table, such as position, absolute arrival time, travel time, and additional data for labeling the captured photons. This enables a flexible reconstruction of images and films from the same data according to freely selectable criteria (Video 1).

Applications

Fluorescence lifetime imaging (FLIM) and Förster resonance energy transfer (FLIM-FRET)

Video 2: Auramine crystallization (see Fig. 6 for description).

The crucial difference to intensity-only imaging is additional contrast FLIM adds arising from differences in the fluorescence lifetime of the fluorophores. This makes information accessible that would remain hidden in a purely spectral intensity analysis. In microscopy, FLIM is mainly used to extract functional information from the differences in the exponential decay rates of fluorescent dyes. Since the fluorescence lifetime of a dye is affected by environmental changes in its immediate neighborhood, it is also a kind of a quantum-sensor of changes in pH, viscosity, and ion concentrations. In principle, fluorescence lifetime imaging can be performed using confocal laser scanning microscopy (CLSM), 2-Photon-microscopy, or wide-field microscopy, whereby the former two provide sharper images due to the confocality, but the use of a wide-field microscope proves to be significantly gentler on living samples.

Here, we present two examples of the use of the LINCam for FLIM. The first example shows lifetime imaging of a lily of the valley slice sample, which usually serves as a standard in microscopy (Fig. 4).

Another example of a FLIM-FRET application comes from immunology, where, after activation, the distance between two domains within a biosensor molecule in T-cells is to be determined. Clear differences can be seen between the pure transfection of the donor dye in T-cells (Fig. 5a) and a mixture of donor and acceptor dyes (Fig. 5b) compared to the donor-acceptor pair coupled to the biosensor (Fig. 5c).

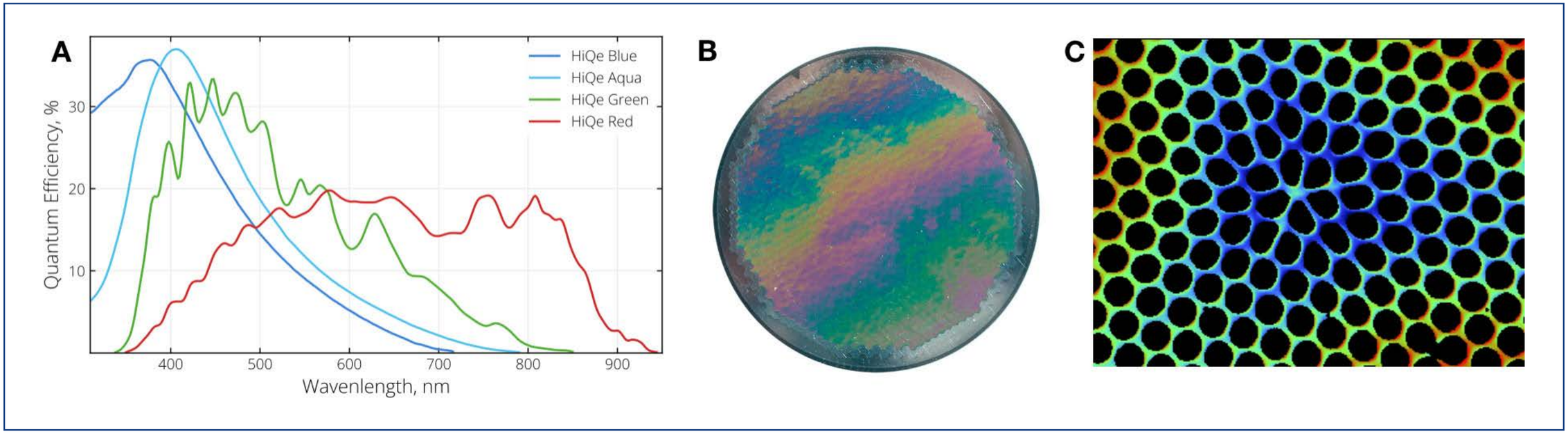


Fig. 3: A: Quantum efficiencies of different photocathodes used in LINCams. The photocathodes are optimized for various applications in different spectral ranges. B: The image shows a multi-channel plate with millions of individual pores. C: The scanning EM image shows an area of the MCP with individual pores. In this case, the pores each have a diameter of 10 µm.

Auramine O crystallization

To demonstrate the applicability of the detection system for video-rate FLIM imaging in chemical reactions [5], we showed the changes in fluorescence lifetime upon transition of Auramine O crystals into the liquid phase after methanol addition and upon recrystallization after solvent evaporation (Fig. 6, Video 2). Auramine O is a diarylmethane dye (maximum absorption: 431 nm, maximum emission: 499 nm) to stain acid-fast bacteria. In the solid phase the crystals show a multiexponential decay with long components. After the addition of methanol, a gradual shortening of the lifetime is observed until the Auramine O crystals have completely dissolved. In the dissolved state, Auramine O has an extremely short lifetime. Due to this short mean lifetime of < 100 ps, it is often used to determine the instrument response function (IRF) in FLIM experiments with fluorophores in the blue-green spectral range. Evaporating of the methanol creates new crystals of Auramine O over time and the lifetime increases again.

3D FLIM with LINCam

In order to generate high-quality optical sections with a wide-field microscope, it can be either upgraded with a spinning disc system or a light sheet illumination, which illuminates a thin layer of the sample perpendicular to the detection light path.

Both imaging techniques were successfully tested on different microscopes with the LINCam. Here, we show an example of a 3D dataset of a cleared rat fetus taken with a light sheet microscope (Fig. 7, Video 3). The transparent specimen, which was stained with Azilarin red to show the skeleton, contains numerous structure-specific autofluorescence signals in addition to the staining. The animated 3D stack consists of 2,080 optical layers acquired at 4 µm step size with an exposure time of 20 s per layer.

For the intensity-weighted 3D lifetime representation, mean lifetime estimates were first created for each slice. Individual pixels were then sorted into a lifetime histogram with different temporal bin widths using Matlab (Mathworks).

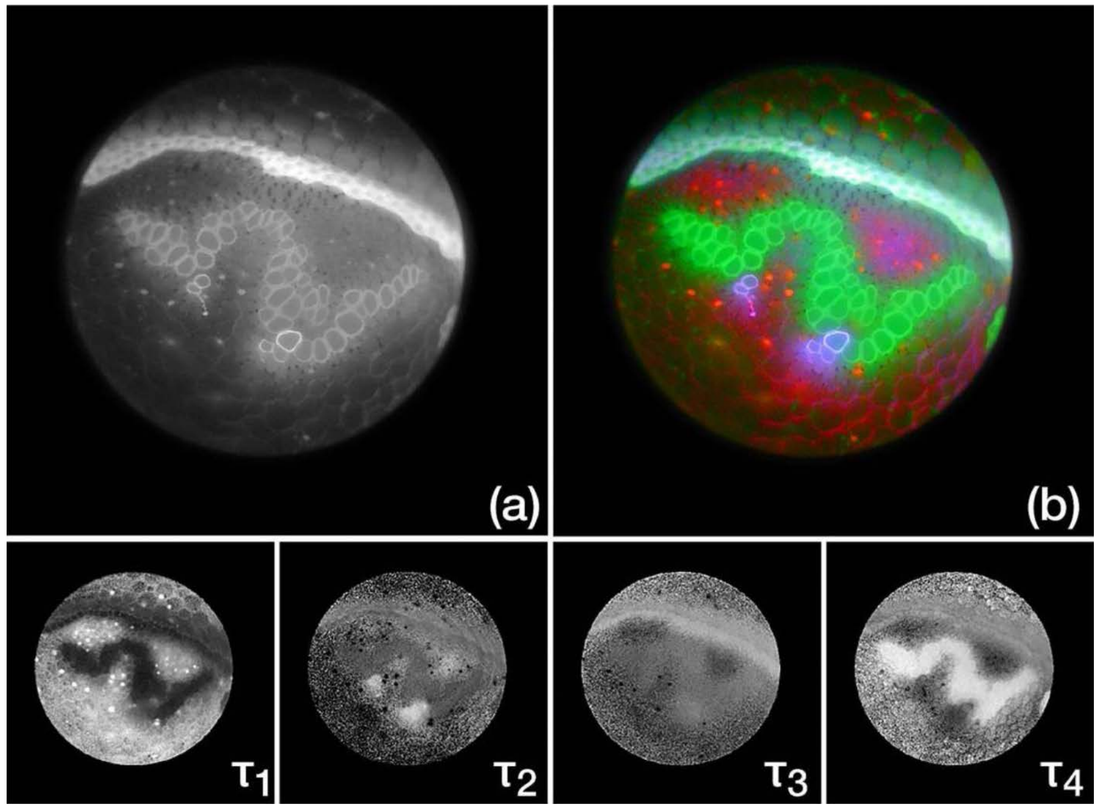


Fig. 4: Intensity and lifetime image of Lily of the valley. The intensity image (a) is a histogram of the positions of acquired photons. Lifetime analysis by multi-exponential maximum-likelihood estimation with our detector system reveals four lifetime components: τ1 = 0.19 ns; τ2 = 0.67 ns; τ3 = 1.95 ns and τ4 = 3.75 ns. The resulting overlay image of the intensity image and average lifetime is shown in (b).

Binary images of all layers were then created, each containing only one lifetime. This was repeated for all lifetimes. From the intensity image, the intensity of the respective lifetime voxel was determined layer by layer for all 2,080 optical slices and saved together with the associated lifetime class as an intensity-weighted lifetime stack in the form of a color channel (i.e., with a lifetime-specific LUT). In this way, a 3D data set with different intensity-weighted lifetime channels (see caption) was finally created and visualized using a 3D imaging program (Imaris, Oxford Instruments).

Single-molecule detection of spectrally similar dyes

Fluorophores such as Cy5, Atto 655, and Atto 647N are almost impossible to resolve spectrally but are characterized by significantly different lifetimes. FLIM is therefore the method of choice for enhancing the number of detectable fluorophores in a sample.

Both the confocal LSM technique with point detectors and time-correlated single photon counting using wide-field microscopy are suitable for distinguishing dyes by lifetime imaging. Compared to wide-field microscopy, however, CLSM has the disadvantage that it has a lower photon yield and a 2-3 times lower acquisition speed [6,7]. Moreover, Single photon counting with the LINCam has the advantage that the data acquisition works virtually without background and readout noise [6,7]. However, earlier photocathodes had a very low quantum efficiency in the red spectral range, which is why they have only rarely been used for single-molecule observations. Yet, thanks to a new generation of very sensitive photocathodes with a quantum efficiency of >15% at 800 nm, detectors are now available that also provide very good results in the red to near-infrared spectral range (Fig 3A).

To demonstrate the usability of the LINCam for the time-resolved separation of spectrally similar dyes, the above-mentioned DNA-coupled dyes were first recorded individually on a glass surface and their fluorescent lifetimes were calculated with a precision of less than 0.2 ns (Fig. 8). The dyes were then mixed and measured again on a glass surface (Fig. 9). It was shown that all dyes were clearly identifiable in the mixture by their lifetime.

Metal-induced energy transfer (MIET) using wide-field microscopy

In Förster resonance energy transfer (FRET), an excited donor molecule transfers energy to an acceptor molecule. In MIET technique, a metal plasmon surface acts as an acceptor, allowing distances to be measured with nanometer precision [8]. The general MIET principle is shown on Figure 10.

André Weber

André Weber studied physics at Otto-von-Guericke University Magdeburg, Germany, and has been a scientist in the microscopy group of the Combinatorial NeuroImaging Core Facility since 2011. He designs setups and concepts for TCSPC experiments and analyses. Through his work on dynamics in biological systems, he became an expert in label-free NADH/FAD autofluorescence imaging of immune, stem cells, and neurons.

To demonstrate the MIET technique with the LINCam, T-cell lymphocytes transfected with a GFP-tagged protein kinase (GFP-Lck) were fixed on gold-coated coverslips. First, measurements were performed under standard epifluorescence illumination conditions with an excitation wavelength of 488 nm. The resulting intensity-weighted mean lifetime images (Fig. 11a) and the intensity images (Fig. 11c) showed the typical lifetime and gray value range for GFP with a slight attenuation at the contact points. The second measurement of the same cells was performed with TIRF illumination and revealed a much higher lifetime contrast (Fig. 11b) related to the MIET effect.

To distinguish fluorescence lifetime components, we performed Maximum Entropy Method (MEM) analysis (Fig. 13). The results of per-pixel amplitude fitting based on MEM analysis are shown in Figure 12.

The amplitudes corresponding to the 0.38 ns and 0.82 ns lifetime components were summed in the intensity image (Fig. 12c) and the amplitude-weighted lifetime map (Fig. 12a). The results for two other components with lifetimes of 2.29 ns and 5.58 ns are also shown (Fig. 12b, 12d). The short component pictures show the typical cluster formation of the protein kinase along the plasma membrane after T-cell receptor stimulation.

Dr. Stefan Richter

Stefan Richter completed his Ph.D. in Physics with a focus on quantum optics. Since 2022, he has been employed by the Combinatorial NeuroImaging Core Facility at Leibniz Institute for Neurobiology, Magdeburg, Germany, where he built a hybrid single photon counting detection system for simultaneous label-free fluorescence lifetime imaging microscopy of NADH and FAD autofluorescence in combination with Raman spectroscopy.

Dr. Yury Prokazov

Yury Prokazov received his Ph.D. in Engineering from Otto-von-Guericke University Magdeburg, Germany, in 2011. From 2005 to 2017 he worked on the development of quantum detectors in the Combinatorial NeuroImaging CoreFacility of the Leibniz Institute for Neurobiology (LIN), Magdeburg, Germany. In 2013 and 2017, his work was awarded the Hugo Junkers Prize for the most innovative basic research and applied research. Since 2017, he switched to the start-up company Photonscore, where he works as managing director.

Metabolic imaging of NADH and FAD autofluorescence

The main interest in observing autofluorescence in cells and tissues lies in their potential for medical diagnostics and in the possibility of examining mechanisms of molecular interactions and signaling processes under natural conditions without the need for the addition of exogenous reagents and performing complex staining processes. To this end, the most important intrinsic fluorophores include amino acids, nicotinamide adenine (pyridine) dinucleotide (NAD(P)H), flavins, porphyrins, melanin, lipofuscin, collagen, and elastin [18–20].

NAD(P)H and flavin adenine dinucleotide (FAD) are coenzymes that are involved in numerous metabolic processes and whose fluorescence intensities and fluorescence decays can provide direct information about the metabolic state of cells [20–22].

The fluorescence intensities of FAD and NAD(P)H depend on the redox state of the tissue. While the oxidized form NAD does not fluoresce, NAD(P)H is fluorescent in its reduced form. With FAD it is exactly the opposite, i.e., FADH2 is not fluorescent while the oxidized form FAD is fluorescent. The ratio of the fluorescence intensities of FAD and NADH thus changes depending on the metabolic state [20].

Dr. Werner Zuschratter

Werner Zuschratter holds a senior scientist position at Leibniz Institute for Neurobiology, Magdeburg, Germany. He has headed the Special Laboratory for Electron- and Laser Scanning Microscopy since 1992 and was coordinator of the Combinatorial NeuroImaging Core Facility of the Leibniz Institute for Neurobiology (LIN), Magdeburg, Germany, between 2012 and 2020. He has extensive experience in modern microscopy techniques with a focus on fluorescence-lifetime imaging microscopy (FLIM) and label-free metabolic imaging.

Video 3: Animation of a 3D FLIM image stack from a cleared rat fetus by lightsheet microscopy (see Fig. 7 for description). [Collaboration with O. Kobler, Combinatorial NeuroImaging Core Facility, LIN].

NADH is mainly involved as an electron/proton carrier in glycolysis in the cytoplasm and in the tricarboxylic acid cycle (TCA) and electron transport system of the mitochondria. It can therefore principally accumulate in both the cytoplasm and the mitochondria, with oxidative phosphorylation in the mitochondria being the preferred route under normal aerobic conditions, since the net yield of ATP is much greater here (30-34 molecules ATP/glucose molecule) compared to glycolytic metabolism (2 molecules ATP/glucose molecule). A shift in NADH distribution between cytoplasm and mitochondria is therefore an important indicator of changes in cell metabolism [18, 20–22].

The observation of metabolic changes in living cells using autofluorescence represents a major challenge for any detection system since the changes to be expected are usually minimal. In addition, the quantum yield of NADH is very low [21]. Therefore, an extremely sensitive, low-noise acquisition system is required, otherwise, the subtle metabolic changes will be lost in the background noise.

On the other hand, assessing metabolic changes by FLIM is facilitated by the fact that NADH has different mean lifetimes depending on whether it is free in the cytoplasm (<0.8 ns) or bound to protein (>1.2 ns). The reverse is true for FAD: bound FAD has a very short mean lifetime (ps - ns range) and free FAD has a relatively long mean lifetime (>1-3 ns) [18, 21, 23]

An increase in the bound NADH fraction (with longer lifetimes) and a decrease in the bound FAD fraction (increase in lifetime) thus indicates a switch from glycolysis to oxidative phosphorylation, which may be an indicator of increased activity, oxidative stress, fatty acid synthesis, and ROS production [22, 24].

In contrast, a shift in the ratio of bound to unbound NADH would be accompanied by a significant change in fluorescence lifetime to shorter lifetimes indicating a shift from oxidative phosphorylation to glycolytic metabolism. Such a shift to reductive glycolytic metabolism is known, for example, from inflamed and carcinogenic tissue [22, 24, 26], which is why an analysis based on fluorescence lifetime can be used for tumor diagnostics, while differences would hardly be recognizable with a purely intensity-based detection system.

To show how well the LINCam system is suitable for metabolic imaging of NADH and FAD autofluorescence, we performed pharmacological stress tests with different cell systems [9, 10] and observed changes in the metabolic state as a function of electrical activity in neuronal cell cultures.

To date, cellular stress tests have been carried out mainly with cell suspensions in a microwell plate, with the mitochondrial function being determined as an average value by measuring the oxygen consumption rate [27, 28].

For this purpose, after recording a respiratory baseline, various modulators of the electron transport chain (ETC), i.e., oligomycin carbonyl cyanide-4 (trifluoromethoxy) phenylhydrazone (FCCP), rotenone, and antimycin are added one after the other [27, 28].

In contrast to the cell suspension stress test method, which relies on a certain minimum number of cells in the microwell plate and only displays an average value, with the LIN-Cam the behavior of individual cells and cell groups can be tested under visual inspection using the same stress protocol mentioned before. With our method, we found interesting differences between mouse strains and tissues as well as between individual cells, indicating different metabolic states within the same cell population. In this respect, the scalability of the camera system proves to be a great advantage, since it enables high-resolution microscopic and mesoscopic images as well as macroscopic observations of larger tissue (Fig. 14).

Application of NADH and FAD autofluorescence imaging could also be used to monitor neuronal network activity and to detect changes in the metabolic activity of individual neurons after electrical stimulation (Fig. 15; Video 4). Subsequent fixation and immunocytochemical staining allowed later assignment of the metabolic signature to subcellular organelles. Moreover, in neuronal cell cultures grown on multi-electrodes intrinsic electrical discharge patterns could be directly correlated with energy expenditure (Fig. 16). In such experiments, we found that immediately after a burst activity (Figs. 16c, d), the intensity of NADH autofluorescence decreased (Fig. 16a) while its fluorescence lifetime increased (Fig. 16b). This means that the concentration ratio NADH/NAD has shifted in the direction of non-fluorescent NAD and the energy consumed came primarily from unbound NADH. The fact that such small metabolic changes are already detected in a neuronal network after spontaneous burst activity emphasizes the sensitivity and the good signal-to-noise ratio of the method, which allows to see changes in photon statistics and lifetime even with fluorophores of low quantum yield and under low light conditions.

Video 4: Imaging of changes in metabolic activity in individual neurons following electrical stimulation combined with immunocytochemical staining after fixation. [Collaboration with E. Altun (OvGU Magdeburg), R. Herrera-Molina (LIN)].

Fluorescence lifetime-based calcium imaging

In addition to label-free recording of autofluorescence, metabolic imaging of cells, cell populations, and tissues can also be performed by injecting calcium-sensitive dyes. Calcium is a very important intracellular messenger for investigating cell-cell interactions in (neuronal) networks and for tracking small intracellular changes in individual cells. Its distribution in neurons and astrocytes generates waves and varies with age and previous activity.

Accurately tracking the spatiotemporal distribution of [Ca^{2+}] with Ca-sensitive dyes is a major challenge, especially since the cellular uptake of calcium dyes can be very heterogeneous in a cell population and the local uptake dye concentration consequently affects the sensitivity and accuracy of the measurement. Fortunately, there are calcium dyes such as Oregon Green 488 BAPTA1-AM (OGB) that are sensitive to free [Ca^{2+}] in the nanomolar range and show changes in fluorescence lifetime upon binding to calcium [29].

In contrast to intensity measurements, lifetime measurements are independent of the intracellular dye concentration. Therefore, fluorescence lifetime can be used to more accurately determine calcium concentrations and to monitor peaks and transients in individual neurons and populations [30] since both the lifetime and intensity of OGB fluorescence increase while bound to calcium. Consequently, depending on the concentrations of OGB and calcium, cells can exhibit the same intensity but different fluorescence lifetimes, allowing measurements of changes in calcium concentrations in the nanomolar to micromolar range.

Here, we showed the intensity and lifetime changes of OGB fluorescence in neuronal-astroglial co-cultures during spontaneous activity (Figs. 17, 18). Fluorescence lifetime changes in the subsecond range can be monitored with the LINCam over a long period of time without bleaching effects.

Raman spectroscopy

Recently, we expanded our microscopic setup for measuring metabolic activity with an imaging spectrometer. With this, it is now possible to simultaneously record NADH and FAD autofluorescence together with time-re-

solved Raman spectra using three synchronized LINCams in combination with multiplexed laser excitation (Fig. 19).

Typically, Raman spectra are superimposed by a predominant fluorescence light, which often masks the distinctive Raman fingerprint regions essential for characterizing various materials. However, given that the Raman process occurs extremely rapid (<1ns), it's theoretically feasible to differentiate the swift Raman photons from the comparatively delayed fluorescence photons using temporal gating. In this context, the LINCam with its ultra-high temporal resolution of less than 50 ps is ideally suited [12]. Leveraging its event-based data structure, the Raman photons can be distinctly separated from the fluorescence decay. This distinction enables the isolation of spectra, significantly enhancing their visibility. Here, we demonstrated the sensitivity and advantage of time-resolved Raman spectroscopy on a test sample of ethanol in highly fluorescent rhodamine B (Fig. 20). In practice, however, the method could be of particular relevance for medical diagnostics, since it could be used to improve the analysis of Raman spectra to characterize various cell tissues, which would be particularly useful in cancer research.

Summary

Current fluorescence microscopy is far from being a non-invasive imaging technique, as most systems require relatively high light doses, which often result in significant photodamage when observing living specimens.

As an alternative, we present the development and application examples of a wide-field imaging method that enables time-resolved image acquisition with an ultra-sensitive detector. The method is based on a single photon counting position sensitive detector, which due to its extraordinary signal-to-noise ratio enables imaging at very low-light conditions, and allows the generation of diffraction-limited images using positional and timing information of individual quanta of light acquired sequentially and recorded as a continuous data stream.

The presented imaging method closes a gap in the field of low-light imaging, where it represents a disruptive alternative to conventional CCD camera. In addition, the method is an alternative to conventional confocal scanning-based fluorescence lifetime imaging.

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The method enables long observation times of sensitive samples and can be used for Ca imaging, single molecule detection, FLIM-FRET applications, and quantum optics, among others. In addition, it also allows label-free imaging of metabolic processes by recording the autofluorescence of various metabolites.

This opens up new perspectives for examining the vitality of cells and tissues and for distinguishing between healthy and pathogenic tissue in medical diagnostics. In addition, the method can acquire multimodal data from multiple detectors simultaneously and can be extended with a spectrometer to a hyperspectral time-resolved Raman FLIM imaging system.

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