Albert Einstein College of Medicine
Bronx, NY
Lucas Sjulson, Mats Nilsson
$1,000,000

Fluorescence microscopy is a foundational tool of the biomedical sciences because it can visualize almost any molecule of interest. However, traditional fluorescence microscopy is limited to 6-7 colors, far fewer than many applications require. This limitation is imposed not by optics, but by the unavailability of a large panel of dyes with distinct colors. A team from the Albert Einstein College of Medicine and Stockholm University will develop FRAINBOW (Fluorescence Resonance-Assisted IdentificatioN Based On Wavelength), an innovative technology that can generate up to 128 spectrally unmixable colors. The project aims to visualize large numbers of diverse mRNA molecules in thick, clarified brain tissue sections, with the potential to revolutionize the understanding of the functional roles of distinct cell types. Two FRAINBOW-based methods will be developed: PUFRFISH (Probes Unmixed in First Round Fluorescence In Situ Hybridization), which can image 128 distinct mRNAs, and PUMAFISH (Probe Unmixing Multi-round Analog FISH), a multi-round imaging technique with theoretical full-transcriptome capacity. The team will also develop user-friendly software tools and an open-source microscope upgrade module to ensure these methods are accessible to the entire community. Successful completion of this project has the potential to revolutionize not only multiplexed RNA FISH, but any fluorescence-based application in the biomedical sciences, from flow cytometry to cancer diagnostics.

Oregon State University
Corvallis, OR
Edward Brook Christo Buizert
$1,200,000

Cores drilled deep in the Antarctic ice sheet provide exquisitely detailed records of past environmental conditions and atmospheric composition going back 800,000 years. These records form the foundation of much of our understanding of natural climate change during this time, such as the Ice Age cycle. There is an urgent need to extend the ice core record further back in time to understand the climate dynamics of earlier periods. Targets include the emergence of Ice Age cycles in the early Pleistocene (2.6 to 1.2 million years ago) and past natural warm periods that provide an analogue for Earth's warmer future. The Center for Oldest Ice Exploration (COLDEX), led by Oregon State University, has recently recovered Earth's oldest pristine ice samples, up to 4 million years old, from the Allan Hills region in Antarctica. Over time, the layers of ice have strongly compressed and

folded over one another, leaving a discontinuous historical record that precludes traditional methods of interpretation. This work seeks to answer fundamental questions about past natural climate cycles by developing and applying new methodologies to decipher old ice from the Allan Hills. The new tools would (1) determine the “arrow of time” of individual ice segments; (2) determine the relationship between global Ice Age cycles, Antarctic climate, and changes in incoming solar energy during the early Pleistocene; and, (3) determine if and how changes in greenhouse gases impacted those early Ice Age cycles. Antarctic ice is the only Earth material that can answer these questions, yet the complications of this folded archive make deciphering it a challenging, high-risk endeavor.

Purdue University
West Lafayette, IN
Niranjan Shivaram, Chris H. Greene
$1,200,000

A team of researchers from Purdue University plan to generate high photon rate, ultrabroadband extreme ultraviolet and soft x-ray entangled photons (biphotons) via two-photon decay of metastable helium atoms and helium-like ions, and then use them in attosecond pump-probe quantum spectroscopy of atoms and molecules. The theoretical part of the proposal will develop a framework to model the generation of these biphotons and their interaction with atoms and molecules, providing guidance to experiments. Entanglement is a uniquely quantum mechanical phenomenon that is at the heart of quantum information science, quantum sensing, quantum enhanced imaging/spectroscopy and other emerging quantum technologies. Entanglement of photons plays a key role in many areas of basic and applied research that leverage quantum advantage. The phenomenon of quantum entanglement in the context of photon-molecule interactions can yield insights into molecular quantum dynamics and provide enhancements in metrology to study ultrafast dynamics on the attosecond (10^{-18} s) scale. Though entangled photons (biphotons), typically generated via spontaneous parametric down conversion (SPDC), have been widely used for decades, their wavelengths have been limited to ultraviolet, near infrared and longer, with limited bandwidth. X-ray biphotons have been generated from SPDC using synchrotron x-ray sources but currently have very low photon generation rates. Further, the experimental application of biphotons to study ultrafast time-resolved quantum phenomena has been limited to two-photon absorption studies with rarely any time-resolved information extracted regarding ultrafast dynamics. The development of classical attosecond light sources to study electron dynamics on their natural attosecond time scale has recently been recognized with a Nobel Prize in physics in 2023. In this project, the researchers will combine ideas from atomic physics, attosecond science and quantum optics to develop an entangled photon source for attosecond spectroscopy. If successful, this source can provide a new path for spectroscopy on the attosecond time scale and beyond.

University of California, Los Angeles
Los Angeles, CA
Steven Jacobsen
$1,300,000

A fundamental problem in eukaryotic biology is how transcription factors and other proteins gain access to DNA, given that DNA is wrapped around chromatin proteins within nucleosomes. For example, genome editing approaches rely on CRISPR-Cas nucleases that must gain access to specific DNA target sequences to induce sequence changes. However, CRISPR-mediated genome editing is highly inefficient at DNA sequences that are tightly associated with nucleosomes. This work aims to tackle this problem by developing fusion proteins that can efficiently edit nucleosomal DNA in plants and other organisms. Planned experiments revolve around a key discovery made recently by our laboratory, that fusion of certain protein domains to CRISPR or to zinc finger systems can cause massive accumulation of the fusion proteins at target genomic sites, while sequestering these proteins away from non-target chromatin sites. This discovery will be leveraged to design and test new CRISPR-based editing systems that are more efficient and specific. The experiments will be done in *Arabidopsis* protoplasts, which will facilitate fast iterative testing of many fusion constructs. Successful editing systems will also be tested in fission yeast cells to show they can be used in a wide variety of eukaryotic organisms. If successful, these potent genome editing tools that could have a major impact on basic research, as well as applied areas such as agricultural improvement.

The University of Texas at Austin
Austin, TX
Edoardo Baldini, Allan H. MacDonald, Vinod Menon
$1,100,000

A cornerstone of quantum electrodynamics is that the seemingly empty vacuum is filled with fluctuations of the electromagnetic field. Understanding the extent to which these vacuum fluctuations influence the emergence of quasiparticle coherence and entanglement in quantum matter is an open question. Theoretical predictions have shown that precise manipulation of the vacuum-matter coupling can lead to selective changes in microscopic interactions and the stabilization of novel quantum phases. This approach relies on embedding quantum materials into optical cavities designed to operate in the dark under a tailored vacuum field. However, to date, this synthetic design of coherent and quantum-entangled states of matter by vacuum fluctuations has remained unrealized. A team of investigators from the University of Texas at Austin and the City College of New York aims to develop novel cavities that realize ultrastrong vacuum-matter coupling at terahertz frequencies and promote macroscopic instabilities toward coherent and quantum-entangled states of matter. Their research will focus on atomically thin materials and their moiré superlattices, with the goal of enhancing their superconducting and fractional Chern insulating states. The PIs will characterize the vacuum-modified phases by studying their low-energy electrodynamics with a suite of advanced microscopy tools. This

activity will be supported by a vigorous theoretical program at the intersection of condensed matter physics and quantum electrodynamics. If successful, these studies will lead to a paradigm shift in the understanding of emergent matter and open a new field of quantum fluctuations engineering.

University of Washington
Seattle, WA
Devin Schweppe, Brian Beliveau, Keriann Backus
$1,300,000

Multicellular life is defined by trillions of unique cells. Understanding how cells work together and what makes each cell unique is the key to understanding organism function. Using techniques developed to measure nucleic acids (RNA and DNA) in individual cells, recent studies have revealed some of the true scope of cell-to-cell differences that define life. However, proteins, not nucleic acids, are the molecular machines responsible for most cellular functions. A comprehensive understanding of multicellularity requires single-cell quantitative measurements of proteins. State-of-the-art single cell protein detection methods (e.g., mass spectrometry, microscopy, and flow cytometry), are useful for providing single-cell biochemical readouts for modest numbers of cells and specific proteins, but these methods fail to scale. Two key reasons for the failure to scale are the inherent challenges of: (1) performing proteomics on small amounts of material and (2) the inability to amplify proteins. This collaboration between the University of Washington and the University of California, Los Angeles, will establish an entirely new approach to overcome both challenges and achieve high coverage measures of single cell protein abundance. The key innovation of this work combines custom reagents, multiplexed proteomics, and state-of-the-art computational approaches to identify proteomes of single-cells at high-throughput using robust, scalable, and easily accessible methods on par with single-cell RNA and DNA assays. This technology will be used to interrogate how cell-to-cell variability contributes to four important areas that are not amenable to established methods: (1) in drug resistance, (2) cellular differentiation, (3) heterogeneous organoid biology, and (4) primary tissue single-cell proteomics.
