Adam J. Bowman

EDUCATION

Ph.D. Candidate in Applied Physics, Stanford University — 2017 – present

A.B. in Physics, *Summa Cum Laude*, Princeton University — 2013 – 2017
Certificate in Materials Science & Engineering
Senior Thesis: *A Molecular Beam Epitaxy System for Studying Unconventional Superconductivity by Scanning Tunneling Microscopy*

Montgomery Bell Academy, Nashville TN – Salutatorian — 2009 – 2013

FELLOWSHIPS AND AWARDS

PicoQuant Young Investigator Award	2023
Stanford Graduate Fellowship	2017 – present
NSF Graduate Fellowship	2017 – present
Kusaka Memorial Prize, Princeton Physics	2016 and 2017
Manfred Pyka Memorial Prize, Princeton Physics	2015
Intel Science Talent Search, Third Place	2013

RESEARCH EXPERIENCE

PhD Research, Advisor: Mark Kasevich — 2017- present

- Developed new techniques for nanosecond imaging using electro-optic crystals.
- Demonstrated wide-field fluorescence lifetime imaging of single molecules and the first lifetime recordings of neuron activity *in vivo*.
- Contributed to experiments towards realizing multi-pass electron and optical microscopy. Multi-pass techniques promise to significantly reduce sample damage in cryogenic electron microscopy and nonlinear optical microscopy.
- Mentored graduate, undergraduate, and high school students.
- Acted as laboratory and laser safety coordinator for the group. Managed safety trainings and PPE, developed group-specific safety guidelines, and performed regular safety inspections.

Princeton Nanoscale Microscopy Laboratory, Advisor: Ali Yazdani — 2015-2017
Built a small molecular beam epitaxy system. Grew samples including nickel/bismuth bilayers and superconducting monolayers of iron selenide and studied them with scanning tunneling microscopy.

Princeton Laboratory for Ultracold Atoms, Advisor: Waseem Bakr — 2013-2015
Built a laser system for two-photon excitation of Lithium-6 Rydberg atoms including locking electronics, diode lasers, vapor cells, and a frequency doubling cavity.

Optical Tweezers, Advisor: Daniel Marlow — 2013
Built an optical tweezer apparatus and developed a two-week lab experiment for the junior laboratory course at Princeton.

Lecture Demonstrations — 2013-2017
Built a Farnsworth fusor to demonstrate nuclear fusion, neutron detection, and neutron activation in the lecture hall. Also built a ping-pong ball cannon and assisted with lecture demonstrations.

Adam J. Bowman

Vanderbilt Institute for Integrative Biosystems Research and Education 2011-2013
Advisors: John Wikswo and Jody May
Built a plasma-activated chemical vapor deposition apparatus for microfluidic fabrication. Helped to build and operate an ion-mobility mass spectrometer.

PUBLICATIONS

Bowman, A.J., Huang, C., Schnitzer, M.J., and Kasevich, M.A. "Wide-field fluorescence lifetime imaging of neuron spiking and sub-threshold activity *in vivo*." (2022) *arXiv:2211.11229*

Reynolds, J. L., Israel, Y., **Bowman, A. J.**, Klopfer, B. B., and Kasevich, M. A. "Nanosecond photoemission near the potential barrier of a Schottky emitter. " *Physical Review Applied* (2023)

Koppell, S. A., Israel, Y., **Bowman, A. J.**, Klopfer, B. B., and Kasevich, M. A. "Transmission electron microscopy at the quantum limit. " *Applied Physics Letters 120*.19 (2022): 190502.

Bowman, A. J., and Kasevich, M. A. "Resonant electro-optic imaging for microscopy at nanosecond resolution." *ACS Nano* 15.10 (2021): 16043-16054.

Klopfer, B. B., Koppell, S. A., **Bowman, A. J.**, Israel, Y., and Kasevich, M. A. "Fast pulse shaping for a novel gated electron mirror." *Review of Scientific Instruments 92*.4 (2021): 043705.

Israel, Y., **Bowman, A. J.**, Klopfer, B. B., Koppell, S. A., and Kasevich, M. A. "High-extinction electron pulses by laser-triggered emission from a Schottky emitter. " *Applied Physics Letters 117*.19 (2020): 194101.

Bowman, A. J., Klopfer, B. B., Juffmann, T., and Kasevich, M. A. "Electro-optic imaging enables efficient wide-field fluorescence lifetime microscopy." *Nature Communications* 10.1 (2019): 1-8.

Koppell, S. A., Mankos, M., **Bowman, A. J.**, Israel, Y., Juffmann, T., Klopfer, B. B., and Kasevich, M. A. "Design for a 10 keV multi-pass transmission electron microscope." *Ultramicroscopy* 207 (2019): 112834.

Bowman, A. J., Scherrer, J. R., and Reiserer, R. S. "Note: A single-chamber tool for plasma activation and surface functionalization in microfabrication." *Review of Scientific Instruments* 86.6 (2015): 066106.

PATENTS

Bowman, A., Klopfer, B. B., and Kasevich, M. A., "Nanosecond imaging methods using optical modulators." Patent Application US17/898093 (2022).

Bowman, A. and Kasevich M., "Wide-field resonant electro-optic imaging devices and applications." Patent Application US17/153438 (2021).

Bowman, A., Klopfer, B. B., and Kasevich, M. A., "Wide-field nanosecond imaging methods using wide-field optical modulators." Patent Application WO2020106972A1 (2020).

Koppell, S. A., **Bowman, A.**, and Kasevich, M. A. "Optically-addressed phase modulator for electron beams." Patent Application US17/613181 (2020).

Adam J. Bowman

PROFESSIONAL ACTIVITIES

Reviewer for *Nature Communications*, *Optics Express*, and *Applied Optics*
Laboratory and laser safety coordinator at Stanford (5 years)

PRESENTATIONS AND POSTERS

Upcoming: Gordon Conference, Quantum Effects in Biology, Galveston Texas, 2023 (invited talk)
Photonics West, San Francisco, 2023 (talk, **PicoQuant Young Investigator Award**)
Advanced Imaging Methods Workshop, UC Berkeley, 2023 (poster)
Focus on Microscopy 2022, virtual (talk)
Affiliates Meeting, Stanford Center for Image Systems Engineering (poster, **Intuitive Surgical prize**)
Photonics West, San Francisco, 2020 (talk)
Max Perutz Labs, University of Vienna, Vienna Austria, January 2020 (invited talk)
Frontiers in Imaging Science II, Janelia Research Campus, 2019 (poster)
Advanced Imaging Methods Workshop, UC Berkeley, 2019 (poster, **second prize**)
Gordon Conference, Single Molecule Approaches to Biology, Vermont, 2018 (poster)

Previous Research

Fluorescence microscopy allows direct visualization of biological structure and dynamics. However, current techniques remain limited for making quantitative measurements and fail to use all the information available from captured light. During my PhD at Stanford, I applied my background in experimental physics to develop an efficient technique for wide-field fluorescence lifetime microscopy (FLIM) which sets the groundwork for the research I propose to undertake at Salk. This technique will enable quantitative 3D imaging of cellular signaling at high speeds.

The current paradigm of fluorescence microscopy is based on spatial measurements of intensity. A key result of my PhD has been to demonstrate that intensity measurements are not always optimal, and that lifetime measurements can improve the readout of biosensors by suppressing technical noise and intensity artifacts. This was made possible by an all-optical fluorescence lifetime technique I developed, electro-optic FLIM (EO-FLIM), which enables wide-field lifetime capture with high efficiency (Fig. 1) and improves the acquisition speed for FLIM by several orders of magnitude over the state-of-the-art. The technique has enabled the first lifetime readout of neuron membrane potential *in vivo*, and is capable of resolving sub-threshold activity and spike propagation at a 1 kHz framerate. EO-FLIM has also allowed efficient single molecule lifetime imaging [1-3].

EO-FLIM uses electro-optic crystals to rapidly modulate the polarization of light and thereby gate a wide-field image onto a standard camera sensor (Fig. 1). The crystal acts as a polarization switch, directing light from the sample into one of two spatially separated images. Thus, all photons may be captured without loss while nanosecond time information is encoded in the ratio of simultaneous optical intensities at every image pixel in parallel. This approach allows self-normalization of a fluorescence signal, as opposed to standard readout methods that use ΔF/F to reference fast changes to a non-simultaneous average baseline. Many biological sensors fundamentally use underlying FRET or quenching processes that modulate lifetime as well as intensity. EO-FLIM realizes a practical wide-field lifetime readout for fluorescence microscopy and opens the nanosecond time dimension for both quantitative optical measurements and fluorescent probe development.

I developed EO-FLIM at Stanford starting from proof-of-concept. This has involved simulation and assembly of custom electro-optical components, development of efficient drive electronics, and construction of custom single-molecule and lightsheet FLIM microscopes.

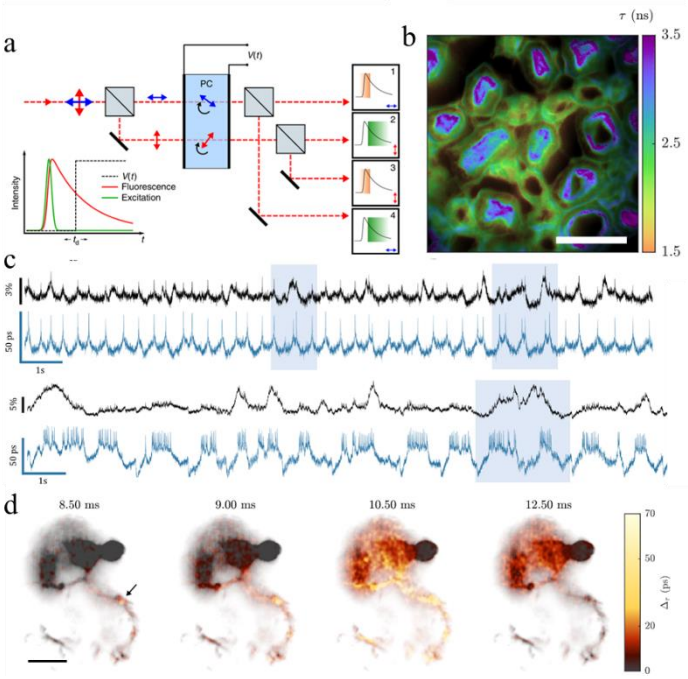


Figure 1: EO-FLIM Technique. (**a**) Schematic of two temporal bin wide-field FLIM with a Pockels cell (PC), illustrated for a single pixel fluorescence decay. Fluorescence emission is first polarized, a time-dependent polarization rotation (step function illustrated) is applied by the PC, and polarizations are split again before the camera sensor. Two pairs of output images correspond to integrated intensity before (1, 3) and after (2, 4) a step function gate is applied in the illustration. The fluorescence lifetime is determined from pixel-wise intensity ratios (**b**) Wide-field FLIM image of a mouse kidney section labelled with Alexa 488 and 568 (scalebar 50 μm). **(c)** Kilohertz intensity (black) and lifetime (blue) readout of a genetically encoded voltage indicator in live *Drosophila* neurons. Lifetime improves signal-to-noise ratio and rejects technical noise and motion artifacts to reveal sub-threshold voltage dynamics. (d) Frames from a time-lapse movie show action potential propagation imaged by fluorescence lifetime (scalebar 25 μm).

Proposed Research

Cells in living organisms communicate across temporal and spatial scales. This communication underlies fundamental processes in biology ranging from the neuroscience of cognition to plant growth and may be mediated through diverse pathways such as membrane potential, signaling molecules, or extracellular vesicles. Microscopy provides a powerful view of the spatial organization of biological matter, but current techniques lack the quantitative, chemical specificity necessary to map signaling throughout complex multicellular organisms and tissues. My proposed research will address this technology gap by developing a platform for quantitative functional imaging in 3D based on fast, volumetric fluorescence lifetime microscopy.

Fluorescence lifetime measures the local environment of a fluorescent probe by sensing its radiative decay rate. This provides many advantages such as being independent of labelling density, photobleaching, and intensity artifacts. However, existing approaches for measuring lifetime have low photon throughput or involve large trade-offs in detector technology, making them incompatible with imaging biological dynamics. To illustrate, current demonstrations of lightsheet microscopy with FLIM detectors require > 5 minutes for a single volumetric acquisition [4, 5].

Fast Lightsheet FLIM Microscopy

I will develop optical technologies for quantitative functional imaging in organisms and tissues. Conventional lightsheet microscopy uses intensity to capture spatial information. By using EO-FLIM, I will provide a wide-field readout for biosensors using fluorescence lifetime. EO-FLIM will allow high-speed lightsheet microscopy without trade-offs in acquisition rate or imaging performance.

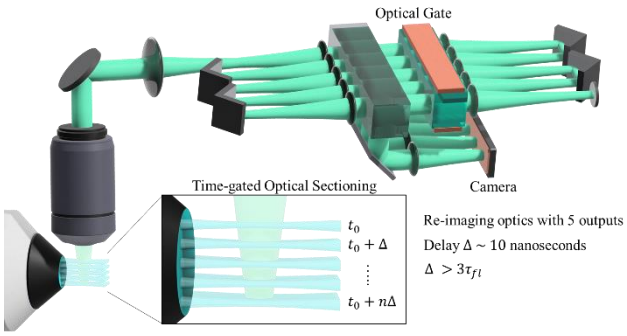


Figure 2: Optical gating microscopy. Planes are illuminated with nanosecond time delay Δt and the resulting fluorescence is optically gated onto spatially separate regions of a camera sensor. A single readout instantaneously captures the sample volume, maintaining optical sectioning.

In addition to FLIM readout, I will also use optical gating to enable volumetric capture at faster rates than are possible with typical lightsheet microscopes, overcoming the speed and sensitivity limitations of sequential scanning. Time-multiplexed acquisition of a sample volume will be accomplished by illuminating each sample plane with a nanosecond time delay as illustrated in Fig. 2. Detection optics will use electro-optic gating and speed-of-light propagation delays to separate these nearly simultaneous slices of illumination. This separation allows the sample volume to be captured by a single exposure of one or more camera sensors. Optical gating microscopy will be well suited to quantitative imaging in moving organisms at up to kilohertz rates. This approach will build on my PhD experience developing EO-FLIM and custom microscopes, particularly the development of nanosecond electro-optic components optimized for imaging. The microscope platform will provide simultaneous advances in both quantitative measurement and high-speed capture that will find broad application to biological problems.

Biological Targets

Quantitative imaging of signaling in multicellular environments promises to reveal how cells communicate and organize to exhibit collective behavior. I will focus on volumetric FLIM imaging of membrane potential in neural circuits as well as simultaneous multiplexing of voltage imaging with complementary lifetime biosensors in cancer tissue and plants.

3D voltage imaging in neurons

FLIM enables stable measurements of absolute membrane potential that are not corrupted by intensity artifacts or photobleaching [6]. Fast volumetric FLIM microscopy of genetically encoded voltage indicators *in vivo* will enable direct observation of functional connectivity in a neural circuit, revealing excitatory and inhibitory relationships between neurons through correlation of upstream spiking patterns with downstream modulation of sub-threshold membrane potential. Within a single neuron, volumetric FLIM will also reveal how dendrites integrate and modify spike inputs in 3D to initiate an action potential. Such studies require both fast, quantitative voltage measurement and kHz volumetric acquisition using optical gating microscopy. This combination has not been possible with current volume imaging methods such as light-field microscopy, which involve fundamental trade-offs between volume acquisition rate, spatial resolution, and sensitivity.

Multiplexed imaging of signaling in tumors and plants

Combination of EO-FLIM with spectral optics will enable multiplexing of voltage indicators with other lifetime probes. These include sensors for calcium, pH, cyclic AMP, neurotransmitters, and most FRET biosensors [7-10]. Multi-dimensional optics (Fig. 3) allow parallel readout of multiple channels of biological information; for example, calcium, voltage, and metabolic indicators can be imaged simultaneously. I will apply multiplexed FLIM to study signaling processes in the tumor microenvironment and in plant tissue.

Cellular ion channels are altered in many types of cancer and play a role in cancer proliferation, signaling, and cell migration [11-14]. Recent voltage imaging studies have established transient and correlated hyperpolarizations in cultures of breast cancer cells [15]. Volumetric voltage imaging in the tumor microenvironment combined with lifetime readout of biosensors will investigate how these findings translate to realistic cancer models (e.g. tissues and organoids) to reveal possible interplay of voltage dynamics with other signaling pathways and metabolism.

Plants are also known to possess excitable cells and a complex set of electrical signals that may play a role in coordinating nutrient response as well as defense against wounds and infection [16]. These range from fast action potentials and calcium waves to slowly travelling membrane depolarizations [17-19]. Cellular plasmodesmata and phloem tissues provide long-range electrical connectivity throughout plants. Plant growth is further associated with calcium influx, oscillations, and membrane hyperpolarization in root hairs [20, 21]. 3D lifetime imaging of plant tissue and roots will provide a quantitative readout of electrical and calcium activity in response to stimuli such as touch, light exposure, wounds, and infection. These studies promise to reveal new insights into how plants sense and integrate information from their environment.

The diverse range of biological questions and model organisms under investigation at the Salk Institute creates an exciting environment to apply 3D FLIM microscopy with maximum impact. I expect that collaborations will have strong feedback into microscope development and will lead to additional directions beyond those proposed here.

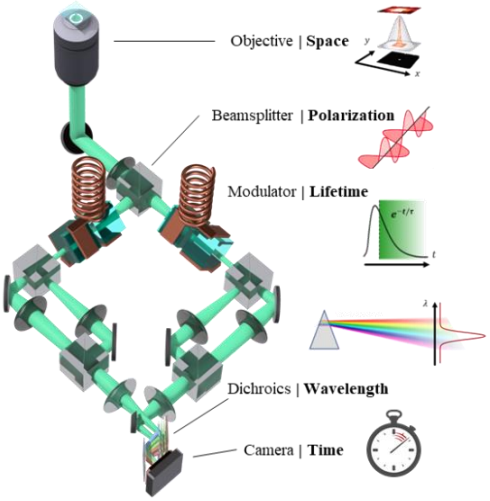


Figure 3: Multi-dimensional imaging optics. EO-FLIM combined with spectral detection enables simultaneous readout of multiple fluorescent probes. Fluorescence is sorted by polarization, time and wavelength without loss onto a low-noise camera. Multidimensional readout optimizes information capture from limited signal and allows computational unmixing of probe signatures.

References

[1] Bowman, A.J., Klopfer, B.B., Juffmann, T., and Kasevich, M.A. "Electro-optic imaging enables efficient wide-field fluorescence lifetime microscopy." *Nature Communications* 10.1 (2019): 1-8.

[2] Bowman, A.J. and Kasevich, M.A. "Resonant electro-optic imaging for microscopy at nanosecond resolution." *ACS Nano* 15.10 (2021): 16043-16054.

[3] Bowman, A.J., Huang, C., Schnitzer, M.J., and Kasevich, M.A. "Wide-field fluorescence lifetime imaging of neuron spiking and sub-threshold activity *in vivo*." *arXiv:2211.11229*.

[4] Hirvonen, L.M., et al. "Lightsheet fluorescence lifetime imaging microscopy with wide-field time-correlated single photon counting." *Journal of biophotonics* 13.2 (2020)

[5] Mitchell, C.A., et al. "Functional in vivo imaging using fluorescence lifetime light-sheet microscopy." *Optics letters* 42.7 (2017): 1269-1272.

[6] Lazzari-Dean, J.R., Gest, A.M., and Miller, E.W. "Optical estimation of absolute membrane potential using fluorescence lifetime imaging." Elife 8 (2019): e44522.

[7] van der Linden, F.H., et al. "A turquoise fluorescence lifetime-based biosensor for quantitative imaging of intracellular calcium." *Nature Communications* 12.1 (2021): 1-13.

[8] Klarenbeek, J., et al. "Fourth-generation epac-based FRET sensors for cAMP feature exceptional brightness, photostability and dynamic range: characterization of dedicated sensors for FLIM, for ratiometry and with high affinity." *PloS one* 10.4 (2015): e0122513.

[9] Lazzari-Dean, J.R. et al. "mScarlet fluorescence lifetime reports lysosomal pH quantitatively." (2022) DOI: 10.5281/zenodo.6363342

[10] Levitt, J. A., et al. "Quantitative real-time imaging of intracellular FRET biosensor dynamics using rapid multi-beam confocal FLIM." *Scientific Reports*, *10*.1 (2020): 5146.

[11] Litan, A., and Langhans, S. A. "Cancer as a channelopathy: ion channels and pumps in tumor development and progression." *Frontiers in cellular neuroscience*, *9* (2015): 86.

[12] Rizaner, N., et al. "Intracellular calcium oscillations in strongly metastatic human breast and prostate cancer cells: control by voltage-gated sodium channel activity." *European Biophysics Journal*, *45* (2016): 735-748.

[13] Pardo, L.A., and Stühmer, W. "The roles of K+ channels in cancer." *Nature Reviews Cancer*, *14*.1 (2014): 39-48.

[14] Sun, Y.S., et al. "Electrotaxis of lung cancer cells in ordered three-dimensional scaffolds." *Biomicrofluidics*, *6*.1 (2012)

[15] Quicke, P., et al. "Voltage imaging reveals the dynamic electrical signatures of human breast cancer cells." Communications Biology, 5.1 (2022): 1178.

[16] Canales, J., Henriquez-Valencia, C., & Brauchi, S. "The integration of electrical signals originating in the root of vascular plants." *Frontiers in Plant Science*, *8* (2018): 2173.

[17] Procko, C., et al. "Stretch-activated ion channels identified in the touch-sensitive structures of carnivorous Droseraceae plants." *Elife* 10 (2021): e64250.

[18] Suda, H., et al. "Calcium dynamics during trap closure visualized in transgenic Venus flytrap." *Nature Plants*, *6*.10 (2020): 1219-1224.

[19] Nguyen, C.T., et al. "Identification of cell populations necessary for leaf-to-leaf electrical signaling in a wounded plant." *Proceedings of the National Academy of Sciences*, *115*.40 (2018): 10178-10183.

[20] Véry, A.A., and Davies, J. M. (2000). Hyperpolarization-activated calcium channels at the tip of Arabidopsis root hairs. *Proceedings of the National Academy of Sciences*, *97*.17, (2000): 9801-9806.

[21] Candeo, A., et al. "Light sheet fluorescence microscopy quantifies calcium oscillations in root hairs of Arabidopsis thaliana. *Plant and Cell Physiology*, 58.7 (2017): 1161-1172.

STANFORD UNIVERSITY
DEPARTMENT OF PHYSICS AND
DEPARTMENT OF APPLIED PHYSICS
STANFORD, CALIFORNIA 94305-4060

MARK KASEVICH
WILLIAM R. KENAN, JR. PROFESSOR
PROFESSOR OF PHYSICS AND
APPLIED PHYSICS

(650) 723-4356
(650) 723-9173 FAX
KASEVICH@STANFORD.EDU

Letter of recommendation for Adam Bowman

To whom it may concern:

I'm excited to recommend Adam Bowman for a Salk Fellow position. Adam has been a PhD in my group for 5 years, focused on developing novel microscopy methods which saturate quantum limits for information extraction from image targets (as described below). I've mentored numerous PhDs and post-docs over the past 30 years. Adam stands out as the best graduate student I've mentored over the course of my career. He is ready now to begin an independent research program.

I recruited Adam into my group to work on a quantum non-demolition electron microscope based on electron probe multi-pass through the sample. Given the very long lead associated with procurement of key subsystems, we decided to pursue optical implementations of the concept. Adam joined the effort, which involved incorporating an imaging, fast switching Pockels cell into a cavity, in order to realize the optical analogue of the proposed electron column. He quickly realized that the Pockels cell switch could be used to implement a novel wide field FLIM microscope. He wanted to switch gears and work on this (I agreed, although in the early days, finding funding was a challenge!). He then proceeded to proof-of-concept demonstrations, to progressively more mature investigations (single molecule, super-resolution FLIM), to our most recent application of the instrument (now 3rd generation and well funded) to voltage imaging of neuron spikes in vivo. His current work is focused on extending the method to light-sheet and two-photon excitation configurations. As the breadth and depth of this imaging program has grown, Adam has been the de facto program leader and has played a central role in developing, writing and winning proposals, recruiting and mentoring team members (grad students and undergraduates), while at the same time innovating apparatus and ultimately making experiments work.

He is a natural instrument builder. The kind of scientist who goes to the Silicon Valley electronics flee markets on weekends to scavenge streak cameras, vacuum tube amplifiers, high voltage switches, …. Or who pours through literature to identify novel electro-optic materials that reduce image distortion and then identifies suppliers and ultimately demonstrates the concept. Who procures a commercial light sheet system on eBay for pennies on the dollar to get inspiration for his subsequent design.

Once built, he gets data out of the apparatus. For example, most recently movies of neural activity. For this work, he implemented the full data analysis pipeline and methodology. He showed excellent judgement in establishing a balance between refined sub-systems (only when needed) and quick implementations. His balanced approach allowed him to quickly identify problems and implement fixes. Finally, instrument built and functioning, he engaged the science. For example, in discovering neuron spike phase locking to external acoustic and/or vibratory stimuli.

I have no doubt he would thrive in the Salk environment and that Salk researchers would benefit from his presence. Please do not hesitate to reach out if you would like to discuss in more detail. Adam is truly a unique scientist. For me, once in thirty years.

Sincerely,

Mark Kasevich

MARK J. SCHNITZER, Ph.D.
Investigator, Howard Hughes Medical Institute
Professor, Depts. Applied Physics & Biology, Stanford University
Ph: 650-725-7438
mschnitz@stanford.edu

Jan. 30, 2023

Re: **Adam Bowman**, applicant to Salk Institute Fellows Program

Dear Salk Colleagues —

I write to recommend Adam Bowman, an Applied Physics Ph.D. student at Stanford, for a Salk Fellow position at the Salk Institute. Adam and I have known each other for about 5 years, and we recently co-authored a research manuscript on fluorescence lifetime imaging of neuronal voltage activity. Adam is a singular young scientist, and I cannot recall ever encountering another applied physics graduate student with a comparable phenotype. He has distinctive qualities and unusual talents, which I will describe here unabashedly so that you can make your own independent judgments about whether he is a good fit for Salk.

In the nearly 20 years that I have been at Stanford, Adam is probably the most naturally gifted experimental physics student that I have met, at least in terms of his ability to conceive of and build novel instrumentation. He started building sophisticated experimental apparatus while still in high school, using his home garage as a laboratory, and came to us at Stanford after having earned his undergraduate physics degree at Princeton. Notably, his recommendations from Princeton were off the charts based on his experimental abilities, even though his scholastic record was average. After matriculating at Stanford, Adam quickly demonstrated his experimental gifts and joined Prof. Mark Kasevich's research group, which specializes in optical metrology. (I was deeply impressed by Adam in his first year of graduate school and tried to recruit him to my own lab, but I think we were doing too much neuroscience to suit Adam's tastes). In the Kasevich lab, Adam invented and built a novel wide-field fluorescence lifetime microscope. This was an impressive feat, which required Adam to design and build his own high-voltage Pockels cells for high-speed electrical control of optical polarization. This in turn required him to identify custom crystalline materials with specific electro-optic and thermal properties. Altogether, there are multiple layers of ingenuity within Adam's instrumentation.

Interestingly, Adam designed and built his microscope without performing formal, computational design studies. Instead, he worked off intuition, leaving me very impressed at Adam's amazing raw talents, which allowed him to succeed notwithstanding his heterodox approach eschewing formal design. Once the instrument was complete, he tried imaging multiple types of biological samples and came to me seeking further suggestions. I proposed that imaging neuronal voltage dynamics based on the lifetime changes of genetically encoded fluorescent voltage indicators could be a good avenue to pursue, and this quickly led to a collaboration with my group. The resulting paper, just submitted for peer review, shows that lifetime fluorescence imaging of neural voltage dynamics in live fruit flies yields superior signal-to-noise ratios than traditional optical readouts of neural voltage activity based on fluorescence intensities.

Overall, I find myself enormously impressed by Adam Bowman's gifts for building instrumentation. While Adam appreciates that biology offers many applications of new microscopes, he has just begun to

survey biological subject matter systematically in search of important problems or sorely needed technical capabilities. Therefore, I think Salk could be an excellent fit for Adam, for several reasons. First, Adam is a wonderfully friendly and easy-going person who gets along well with everybody. You will greatly enjoy working and spending time with him, as have I. Second, my hope is that by immersing Adam within a top-flight biological research environment, Salk will help Adam to realize his full scientific potential. He will gain a greater appreciation for important problems in biology and how his optical inventions can help the field. Third, Adam will contribute a lot to Salk's ongoing biological research programs. He collaborates well with others and will be naturally drawn to help biologists who approach him with technical problems. These interactions will be mutually beneficial.

In summary, Adam Bowman has incredible talents for building instrumentation but would benefit from an environment that guides him toward important scientific challenges. Salk could be the place where Adam unlocks his full potential. If you agree, I enthusiastically endorse a decision to hire him, as I know you will greatly enjoy having him as a colleague.

Sincerely Yours,

Mark J. Schnitzer, Ph.D.

STANFORD
UNIVERSITY

Department of Chemistry
364 Lomita Drive
Stanford, California 94305-4401
Email: wmoerner@stanford.edu

W. E. (William E.) Moerner
Harry S. Mosher Professor
Professor, by courtesy, of Applied Physics
Phone: (650) 723-1727

February 7, 2023

Evaluation and Selection Committee
Salk Fellow Program
The Salk Institute

RE: Recommendation for Adam Bowman

Dear Members of the Selection Committee,

I am truly delighted to provide my unequivocal, very strong recommendation for Mr. Adam Bowman for a Salk Fellow position. If you read no more, let me emphasize that Adam Bowman is simply a superstar in the area of inventive physical concepts to advance biological imaging!

I have known Adam since Spring 2018, when he and his advisor met with me to discuss his clever ideas to develop a widefield microscope to measure lifetimes using a high speed, large aperture, electro-optic modulator. Even at this time, it was clear that he was uncommonly bright, and I really mean this: you may think all students at Stanford are bright, but I would say many are very good, while a very few truly stand out. Adam is of the latter class. A year later, Adam was a student in my advanced graduate student class on "Single Molecules and Light", and he was far above all the other students in his immediate understanding of the material I was presenting coming from the fields of Chemistry, Applied Physics, Bioengineering, and Biophysics. He gave a superb class presentation on the highly complex method named MINFLUX (from Stephan Hell's lab), and explained it all, including the calculations of Cramer-Rao lower bounds! This talk was so good that I had him present to my own group, in the hope of inspiring them! I have also been a member of his PhD thesis committee in the Physics Department here at Stanford, and his 4th year presentation of his research progress was on the level of that from a postdoc with several years of experience. You can see why I say he is really stellar.

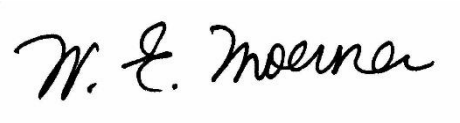
Adam has been actively pursuing his wide-field fluorescence lifetime imaging microscopy (FLIM) ideas, not only with continued very clever inventive new designs with higher and higher performance, but also with direct demonstrations from single molecules to cellular imaging. Of note, the collaborations he has developed extend beyond the physical experts to include members of the biomedical community, a very useful quality that I value highly. My students and I find him highly collegial, and he quickly communicates his enthusiasm for his latest amazing machine.

Adam Bowman has demonstrated in multiple ways that he is a highly talented inventor, bubbling with ideas. He is just unstoppable when he wants to solve a problem. I know that when he needed very high rf electric fields for the elector-optic modulator, he found the amateur radio rf amplifiers he needed, designed the required water cooling, and built exactly what he needs! He

has also amazed me by his pursuit of eBay deals and special new electronic devices, like a nano-sized vector network analyzer. He is an immensely talented physical scientist with a razor-sharp focus on advancing biological imaging and discovering new biology.

Considering future research plans, I am simply inspired by the breadth and depth of mature ideas he presents to achieve quantitative imaging of biosensors and FRET (including voltage sensors) using lightsheet EO-FLIM. He also has more ambitious ideas for fast volume capture with time/frequency domain PC multiplexing. These capabilities are not available with the same performance in other approaches, and the results from Adam's devices will have high impact on the understanding of biological interactions and cellular patterning. Bowman's work should fit in very well with Salk research areas in neuroscience, plant biology, protein interactions, and metabolism. Bottom line, he is a builder par excellence, and not just a builder alone making fancy boxes, but with bioimaging as the challenge to be addressed. I believe that there is much yet to come from Adam Bowman, which is why I feel he is exceptionally qualified for a Salk Fellow position. He is intensely innovative and creative, is a joy in personal interactions, is unfazed by obstacles, is willing to collaborate to push science further, and is driven to make a significant difference in this world through his new tools and his ideas and their applications to the toughest challenges in bioimaging. He is mature and clearly ready to operate an independent lab, and will greatly benefit from an accelerated path to an independent research career. It is worth noting that Salk scientists will be the first to profit from Adam Bowman's novel tools! Taking all these aspects into account, I therefore provide my unqualified and strongest recommendation to you to select him.

Sincerely,



W. E. (William E.) Moerner
Harry S. Mosher Professor of Chemistry
Professor, by courtesy, of Applied Physics
Nobel Laureate in Chemistry, 2014

Personal Statement

Biology presents staggering complexity and diversity across many orders of magnitude in both space and time. My personal motivation is to develop improved measurement techniques that can be applied to a broad class of biological problems. From this perspective, I believe three exciting frontiers are (1) uncovering the neural basis of cognition at organ scale; (2) developing artificial tissue environments to effectively mimic human disease at cellular scale; and (3) cataloging the diversity of the cellular proteome at molecular scale. These three directions are all rapidly advancing, have large potential for impact, and stand to be driven by advances in basic technology and measurement.

The human brain is a remarkably complex organ featuring $\sim 10^{14}$ synapses, yet its function is robust to local perturbations from injury and disease. Understanding how the brain encodes information, the basic processes underlying cognition, and the mechanisms of neural disease promises both fundamental and applied impacts. Ideally, one would like to attach a small sensor to every cell in the brain and measure what it is doing with millisecond precision. Unfortunately, current technology is far from such a comprehensive readout, and principles of information theory imply it may be impossible. Newly developed methods for high-density optical and electrophysiological recordings reveal how ensembles of neurons function locally and across distant brain regions. Parallel advances in scanning electron microscopy and expansion microscopy are providing volumetric maps of cellular connectivity at the synaptic level. Fusion of these functional and structural datasets will yield new advances towards uncovering the basic mechanisms of cognition and disease.

At a tissue scale, improved biological measurements result in immediate applications to medicine and drug discovery. How can human disease be effectively mimicked on a lab bench at the scale of a tumor, an organ, or a multi-organ system with high fidelity? Animal experiments are slow, costly, and often fail in translation. Similarly, conventional *in vitro* biology studies cells in environments that are far from their native states. Microfluidic environments, organs-on-a-chip, and 3D tissue cultures can more accurately simulate *in vivo* conditions while enabling tight coupling between biology, automation, and real-time diagnostics like mass spectrometry. Organ-on-a-chip systems in particular promise to empower high-throughput and quantitative experimentation to expedite drug discovery pipelines and pre-clinical testing.

Finally, an emerging frontier at the molecular scale is measurement of the cellular proteome. Low-cost optical DNA and RNA sequencing has enabled modern genomics. Now, technologies are developing to map the distribution, molecular structure, and interactions of proteins in the cell. Combination of biochemical techniques for protein sequencing with structural studies from cryogenic electron microscopy and AI simulations promise to unlock broad impacts by associating sequence, structure, and function. This work will help to reveal new biomarkers, drug targets, and molecular mechanisms for disease. Further, emerging microscopy methods are poised to improve radiation damage limits for electron microscopy, enabling imaging of smaller protein structures and even single particles. It may eventually prove possible to capture movies of protein folding and dynamics in solution.