Molecular cell biology and advanced microscopy: an interview with Joshua Z Rappoport

Joshua Z Rappoport^{*1}

¹*Northwestern University Feinberg School of Medicine, IL, USA*

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Dr Joshua Z Rappoport, PhD, speaks to Nawsheen Boodhun, Managing Editor. Rappoport completed his bachelor's degree in Biology at Brown University (RI, USA). He then went on to earn a PhD from the Program in Mechanisms of Disease and Therapeutics at the Mount Sinai School of Medicine Graduate School of Biological Sciences of New York University (USA). Rappoport spent the early parts of his career working as a postdoctoral researcher at the Laboratory of Cellular Biophysics based in The Rockefeller University (NY, USA). He was subsequently recruited as a tenured faculty member to work as part of the School of Biosciences at the University of Birmingham (UK). 2014 marked the return of Rappoport to the USA, where he is currently a Research Professor in Molecular Cell Biology at the Northwestern University Feinberg School of Medicine (IL, USA). He is also the Director of the Center for Advanced Microscopy (CAM) and Nikon Imaging Center (NIC), a large core facility consisting of eight members of staff that support around 200 different laboratories.

Could you tell us what encouraged you to embark upon a career in molecular cell biology?

My undergraduate research training was in classical comparative respiratory physiology. My PhD was spent studying cellular urate transport. This is where the transition to molecular cell biology took place. My PhD supervisor, who sadly passed away while I was a postdoc, was an MD nephrologist. She had an amazing perspective linking human disease to protein structure/function, and even human genetics. I have always been interested in the processes underlying health and disease and see molecular cell biology as the ultimate mechanistic scale at which genotype meets phenotype.

Please give us an insight into your career to-date. What would you say has been the highlight of your career?

I spent 6 years as a faculty member running my own research lab, with the school microscopy core as the administrative component of my job. Now I am a full-time core facility scientist and I get to focus completely on the techniques and technologies that support the work of hundreds of

researchers. That transition point, when I moved to my current position, was definitely the highlight of my career.

Your work involves the utilization of advanced microscopy to better understand cell biology. Could you tell us more about this?

As Director of a core facility, I get to work together with researchers from numerous disciplines, helping them solve problems, and assisting them with the most advanced imaging tools available. Two areas of great interest for me are live-cell imaging and super-resolution microscopy. The former allows you to observe as events unfold in real time, and provides significant benefits for statistical analysis. As for super-resolution, this employs extremely innovative techniques to discern structures closer together than the conventional diffraction limit of light microscopy. Most microscopes are limited to a resolution of about 250 nm. The Nikon N-SIM system we have can go down to ~100 nm, and the Nikon N-STORM system can resolve nearly tenfold better than a conventional light microscope. Importantly it is at these scales that many critical structures and processes exist in cells.

I am also heavily involved in the mentorship of more junior core facility scientists and networking among core facilities worldwide. In particular I am very active with the Association for Biomolecular Resource Facilities (ABRF) and the Core Technologies in Life Sciences (CTLIS) association.

In your recently published study, confocal microscopy was used to quantify receptor trafficking [1]. Could you explain the advantages of using this technique?

Conventional widefield epi-fluorescence microscopy is relatively cheap and easy. It is also quite efficient as you collect all the light emitted from the sample. However, this results in significant out-of-focus blur that can prevent locating structures of interest. Many receptors localize to the cell surface prior to stimulation and then when activated they traffic into the cell. It can be very difficult to see this process, unless you have a way to get rid of the out-of-focus blur. Confocal microscopy uses pinhole optics to remove the out-of-focus blur, thus you get clear images that allow you to track the receptors.

What other processes or techniques would you say is ideal for the study of signalling and trafficking of cellular receptors?

Total internal reflection fluorescence (TIRF) microscopy selectively illuminates the adherent cell surface. If you grow cells on a coverslip and then stimulate receptors, using TIRF microscopy you can watch each of the initial stages of the trafficking of active receptors – clustering, internalization and the start of intracellular trafficking. Plus, TIRF can permit very rapid imaging with very little exposure of the cell to the potentially damaging laser light, so it is ideal for live-cell imaging.

Could you share with us some elements of the projects you are currently working on?

As a core facility scientist I spend most of my time helping out my colleagues with their research. This can include issues regarding sample preparation and choice of microscopy system, straight through to image analysis. When we don't have the instrumentation to support potential users, we have to make strategic decisions regarding procurement. Currently we have users who want to combine atomic force microscopy (AFM) with confocal microscopy to measure things such as surface topography and stiffness of specific structures that are fluorescently labelled in live cells and can only readily be imaged with confocal microscopy. Thus I have spent a great deal of time working together with vendors and our user base to demonstrate this equipment with the relevant samples. Correlative multi-modal imaging is something that I have been interested in for a long time, and this combination of techniques has some very exciting potential applications for our users.

What aspect of your work do you enjoy the most?

When I can help our facility users employ new and exciting microscopy tools to get answers to questions that they never thought they would have. Quite often we have users who have been employing conventional confocal microscopy to image their samples, but have questions that

require more advanced techniques such as live-cell imaging or super-resolution. It is extremely satisfying to help people advance their research into new directions through application of cutting-edge techniques.

With regards to 'gaps in the field', could you express what area you feel requires more attention?

Microscopy always involves trade-offs: if you image faster, you will have worse contrast; if you want better resolution, you won't be able to image as deep... However, there are some really exciting new developments in microscopy techniques and technologies that are greatly reducing these types of either/or limitations. Also, it is great to see the solutions that experts in optics are developing in their labs get commercialized and made available for common use.

What would you say is the greatest challenge in your field of work and how does this affect other researchers?

I think that funding is the greatest challenge. Whether it is grants for new equipment or to fund the research projects of our users, there is not enough money made available to fund all of the fantastic deserving research being proposed.

Could you share some advice that can aid early-career researchers?

I think that the conventional research career trajectory (PhD to postdoc to professor) now represents the minority of cases. Therefore, people need to strategize from an early point in their career and ask themselves where they want to be and consider what are still often referred to as 'alternative' career paths – an exciting and heterologous combination of options.

In the next 10 years, what advancement do you hope to see in the field of molecular cell biology?

I think through the use of technologies like genome editing combined with advances in microscopy instrumentation we will soon be

able to image processes in live primary cells with inherently fluorescently tagged proteins expressed at endogenous levels. This has already been accomplished to some extent in certain cases, but the standard is still far behind. Also, although I get exposed to really cutting-edge approaches when I go to conferences or read papers, many people are still overexpressing eGFP fusion proteins in immortal cell lines and imaging them with slow and low-resolution systems. I am very interested in increasing the availability of advanced methods to as many people as possible, and I think this should be a goal for the field in the next decade.

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Address correspondence to: Joshua Rappoport; Northwestern University Feinberg School of Medicine, IL, USA; joshua.rappoport@northwestern.edu

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s.cavana@future-science.com