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SPOTLIGHT

INTERVIEW

Spatial mapping: shaping the future of I-O diagnostics and treatment

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Lauren Coyle, Commissioning Editor, *Immuno-Oncology Insights*, speaks with **Banafshé Larijani**, Director of the Centre for Therapeutic Innovation, University of Bath, about the development and application of a novel spatial mapping technology designed to improve cancer diagnostics and personalize treatment planning.

Immuno-Oncology Insights 2024; 5(4), 95–102

DOI: 10.18609/ioi.2024.015

Q Could you share an overview of your career and what you are currently working on?

BL: I started as a physical scientist and a chemical engineer during my undergraduate and Master's studies in France and Oxford. Afterwards, I pursued a PhD in Molecular Biology and Biophysics, focusing on the biogenesis of the nuclear envelope, at the University of Massachusetts, Amherst College. I completed my postdoctoral studies at Imperial College London before moving to the London Research Institute under Cancer Research UK (CRUK), where I led the Cell Biophysics group for 14.5 years.

Throughout my time at CRUK, I utilized my physical sciences knowledge to develop and implement photonics-based tools, aiming to address questions related to cancer signaling pathways. My focus has been on advancing early diagnostics rather than therapeutics, because there are many ways to deal with this pathology. I collaborated with colleagues at CRUK to develop new methodologies, which led to the development of the spatial analysis and cancer diagnostic tool FuncOmap.

I recently shifted from fundamental research on molecular mechanisms to translational clinical work. Currently, I lead the Centre for Therapeutic Innovation at the University of Bath. By utilizing advances in physical sciences and engineering, we aim to address specific questions in pathologies such as cancer. Our underlying philosophy is precision, and precision science allows us to develop quantitative assays that will hopefully advance personalized medicine.

Q How does spatial mapping improve the accuracy of cancer diagnosis and treatment planning for clinicians?

BL: Before spatial mapping, physical scientists, or spectroscopists, operated in a 2D environment. While we used some specific spectroscopic tools to study the behavior of molecules and how they communicate, we could not determine where these interactions were occurring in 3D space until the advent of spatial mapping technology. These novel techniques leverage semi-quantitative imaging, which became beneficial in visualizing and partially quantifying the locations of numerous molecules within a tissue sample. Other microscopes lack this capability, and even advanced imaging tools such as PET and MRI scans do not resolve to the molecular interaction level.

Single-cell data is typically an inferred result from post-imaging mathematical models based on fluorescence intensity, hence the term 'semi-quantitative'. Commonly, even when quantifying these intensities and localizing multiple markers, information on marker function is lacking. Current commercial spatial mapping tools available in the market can map 40–100 proteins and even genomic events across many markers.

Although semi-quantitative mapping is an improvement from traditional spectroscopic tools that were precise but not localized, simply measuring the amount or expression levels of the proteins and semi-quantifying their location within the tissue does not reveal their actual function, and how they communicate with each other. Only one microscope, located at the University of Strathclyde with Professor Gail McConnell, can map from mm-scale tissue samples down to single cells, roughly 8–10 µm in diameter. The critical interactions in cellular communication happen precisely at this level, encompassing proteome, lipidome, carbohydrates, and genomic material. Further, studying these molecules in isolation does not align with how they function in nature, where such segregation does not exist.

Our objective is to develop a method to quantify the relationship and the mechanistic properties of molecules with one another at the spatial level. This would be transformative for

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early diagnostics, because effective treatment with small molecule drugs ultimately depends on understanding the behavior of a drug towards an array of molecules.

Scaling up to the tissue or whole-organism level is much more complex due to the intricate interactions within these systems. Precision plays a crucial role here by determining how treatment affects the interaction between molecules. Consequently, it enables precise intervention in the treatment and reduces the quantity of treatment required, thereby minimizing side effects and toxicity for the patient.

Spatial mapping has already contributed to these efforts but moving into 3D and higher-dimensional mapping would be a significant advantage. It would allow not only the identification of localization but also the understanding of the characteristics of these molecular interactions within the 3D environment.

Q Looking at FuncOmap, in what ways has spatial mapping contributed to the personalization of cancer treatment, particularly in cases such as clear cell renal carcinoma (ccRCC)?

BL: Even with specific cancers such as ccRCC, there is significant heterogeneity in how the disease presents itself. There is nothing uniform or homogeneous about the pathology. This lack of uniformity is exactly why, 24 years ago, I chose to focus on early diagnostics rather than therapeutics, using the tools my team and I developed throughout my career.

To my knowledge, there still is no specific set of biomarkers that effectively addresses ccRCC. We are still working on the early diagnostics of this cancer type and exploring different signaling pathways. One pathway highlighted in our study involves transcription factors, known as hypoxia-inducible factor (HIF) complexes [1].

One reason why cancer is so complex is because there is no uniform pathway defining its mechanisms. Instead, cancer results from disruptions across a multitude of pathways and interactions. We selected ccRCC as an example due to its status as an unmet need and focused on HIFs in collaboration with Professor Alan McIntyre at the University of Nottingham. The aim was to quantify what we call the 'interactive state'—how one protein interacts with another within this cancer type. By comparing these interactions to healthy kidney tissue, we identified the key differences. We then looked at another set of proteins involved in downregulating immune pathways. Specifically, we studied the impact on the regulation of T cells, aiming to

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prevent dysregulated differentiation. The second class of proteins, ICIs, was also examined for their interaction with HIFs.

Studying such interactions is important because there is a severe lack of oxygen in the TME. This hypoxic condition influences the behavior of transcription factors such as HIFs, impacting the immune system's regulatory mechanisms. ICIs become particularly significant in this context, as these pathways are central to immunotherapy.

In this study we focused on quantifying the interactions between ICIs and HIF complexes by employing spatial mapping, specifically the FuncOmap methodology. This quantification is essential for accurately assessing how these pathways correlate across different tumor regions. Our findings showed that the quantity of these transcription factors does not directly correlate with the tumor's aggressiveness and the interactive states of the ICIs, contrary to what is often suggested in the literature.

By using FuncOmap, it was demonstrated that the functional state of these molecules does not correspond to their expression levels. In other words, high fluorescence intensity of these proteins does not necessarily reflect their interactions.

While spatial quantification of protein interaction is a significant advancement, we are still far from fully understanding these interactions and applying this knowledge to personalized cancer treatments. Looking at the future, one could imagine that, with data from numerous studies across various cancer types, insights from quantitative spatial mapping could play a key role in determining a patient's response to treatment.

Currently, depending on the type of treatment and the cycles of chemotherapy or immunotherapy, it is very difficult to assess the patient's response at a molecular level. For example, after three cycles of a chemotherapy which normally requires eight cycles, we could evaluate the response, which would be extremely helpful for personalizing treatment for the patient. This approach is important for managing side effects, as many treatments are not targeted enough to avoid them, and immunotherapy is a key example. Only a small percentage of patients respond completely to immunotherapy. One of the reasons for this is that the functional levels and interactive states of molecules are not measured at a spatial level—only the quantity of proteins is investigated. No chemical theory or equation has definitively shown that drugs or inhibitors are effective simply by targeting the quantity of a molecule (protein). We must understand what happens when two molecules interact or change their shape, and the drugs produced by pharmaceutical companies need to be assessed for how they perform in a complex, 3D tissue environment.

This is where personalized medicine becomes crucial, because no two people have the same immune environment. It is crucial to understand the patient's immunity prior to any treatment, and this is not yet done systematically. Once true precision is achieved, we will be able to advance toward personalized medicine.

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Q What are the benefits of using FuncOmap for patient stratification and treatment decision-making?

BL: The key benefits come from functional mapping and quantitative investigation of communication between molecules. As I mentioned earlier, it is not the amount of a particular molecule, whether it is a nucleotide involved in genomic events or mRNA coding for a specific protein, that should be the sole focus. The more important aspect is whether the proteins, once formed, are properly communicating with each other. If dysregulation occurs or regulations fail to function properly, it is crucial to map these interactions before treatment and in a much more quantitative manner, which helps to stratify patients more effectively.

Stratifications are often done using binomial systems based on expression levels, and categorized into groups such as 0, 1, or 10, with nothing in between. Stratifying patients accurately is challenging with only three points. For example, think of a simple ruler: currently, the points used to determine whether a patient needs treatment are between 1–10 cm. However, the in-between measurements, such as 2, 3, 4, etc., are not being considered. In our studies, we have shown that implementing a high dynamic range method allows for more nuanced and precise stratification.

Functional mapping creates the necessary dynamic range for effective stratification both at the spatial and non-spatial levels. While we are still far from fully integrating this into treatment decisions, this method, in conjunction with determining the immune environment, is a crucial step. However, no method works single-handedly.

Every precise method, including FuncOmap, has its pros and cons. If we correlate FuncOmap to the immune environment and, eventually, link it to genomic events at the DNA level, we will have three quantitative readouts. In today's computational framework environment, as demonstrated in our paper in collaboration with Dr Julian Padget, this can be automated [1]. Instead of assessing 20 data points, we can now analyze 5,000, enabling more precise decision-making. Nowadays, the majority of high-level data reading can be automated using machine learning.

Statistical analyses are also crucial to investigate large numbers of patients for whether they should be treated with a specific type of drug or not. It is not sufficient to only investigate patients close to the fitted curve produced by statistical methods. The key to moving from precision to personalized medicine lies with the outliers, as they are crucial to understanding why some patients become resistant to different therapies, including immunotherapy.

Spatial mapping can help in this regard, as it enables the investigation of multiple markers as opposed to one or two. All things considered, we are not suggesting that the expression levels of markers, whether nucleotides, proteins, lipids, or carbohydrates, should be erased. Instead, assessing expression levels should be the first step. The next stage involves investigating the immune environment and examining the functional, or interactive, state of the molecules in question. Without this, we risk mistreating patients.

Q How can FuncOmap be applied to other types of cancer, and how do you envision its integration into routine clinical practice?

BL: This type of spatial mapping, whether semi-quantitative or FuncOmap, is not limited to cancer. It can be applied to any pathology, including chronic pain, diabetes, cardiovascular disease, or infectious diseases. We are dealing with how molecules communicate and interact with one another, regardless of the complexity of the disease. Disease complexity can be deciphered by investigating the multiple markers in space, either semi-quantitatively or quantitatively using FuncOmap.

Furthermore, this technology is not only applicable to solid tumors but can also be extended to other types of cancer, such as leukemia. FuncOmap can also be applied at the single-cell level, allowing us to investigate the interactions of these cells, and the markers they express. In essence, this approach offers a comprehensive view of how diseases unfold at both the tissue and single-cell levels, paving the way for more precise diagnostics and treatment strategies across a broad spectrum of diseases.

I am currently working with surgeons, particularly Dr Amanda Kirane at Stanford Medical School within the melanoma team, and they already have the facility to use spatial mapping in clinical settings. Specialized microscopes are currently used to investigate immune environments spatially, but FuncOmap is designed to be more accessible, as it can be implemented on different types of standard microscopes. FuncOmap, licensed by the University of Bath, is essentially an app that can be added to various microscopes, expanding its potential for broader use in clinical settings.

Notably, surgeons have a unique position of authority in the clinical setting. With ethical approval and standard operating procedures, they can collect specimens from patients, which is crucial for implementing advanced techniques such as spatial mapping. By investigating the immune environment and the functional states of molecules before treatment, this tool can be used to refine decision-making around whether a patient will need treatment or what doses are appropriate. Lastly, FuncOmap is not associated with any pharmaceutical company, allowing it to be used more routinely at the academic and clinical level.

Q What excites you most about the potential of spatial mapping, specifically the FuncOmap, in cancer treatment?

BL: It is exciting to see the culmination of 24 years of work coming to fruition. The hard work of many people within my team and contributions from researchers and clinicians, spanning countries such as the USA, France, and Spain, have enabled us to get this far.

I am also thrilled to collaborate with surgeons who think that molecules are as important as the surgical knife. Sometimes the localization of the tumor prevents its removal solely by surgery. For example, some biopsies can be as small as 0.5–1 mm, which requires going down

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to a single-cell level. Working with surgeons willing to incorporate spatial mapping methods into their pathology laboratories, such as Dr Kirane, is very exciting. Even parts of the National Cancer Institute in the USA are starting to notice our work and recognize its importance. Hopefully, in the next 5 years, we can advance to have one or two examples of FuncOmap in a surgical environment.

Q What are the key future perspectives for you in the next 5 years?

BL: In the next 5 years we hope to secure significant funding from the USA, Europe, or the UK to fully implement FuncOmap in collaboration with surgeons. If we can conduct this as a pilot study across three hospitals beginning to work with us, that alone would be quite an achievement. However, whether FuncOmap becomes a worldwide methodology, will depend on the pharmaceutical companies, which is where it becomes more political.

Having worked with the team at Stanford, including Dr Kirane and Professor Jeffrey, I realized that it truly pays off to work with surgeons who are open-minded to exploring molecular and cellular processes. I envision this kind of work as the way forward over the next 5 years, at least for myself and my center. We are beginning to implement these techniques to address questions related to chronic pain, particularly in cancer patients, and gain a deeper understanding of the underlying mechanisms.

In essence, our work is a combination of implementing computational frameworks, understanding molecular mechanisms, and working with surgeons to ultimately benefit the patients. This is how I envision the path forward. They may be small steps, but they are a huge improvement compared to where we were 4 years ago.

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AUTHORSHIP & CONFLICT OF INTEREST

Contributions: The named author takes responsibility for the integrity of the work as a whole, and has given their approval for this version to be published.

Acknowledgements: None.

Disclosure and potential conflicts of interest: The author has received an alumni grant (allocated to the University of Bath). She is also the Academic Editor of *PLOS One*, Chair of the Scientific Advisory Board of IDiBe, University of Miguel Hernandez, and Subject Editor of *Journal of British Cancer Reports Translation Therapeutics*. FuncOmap is licensed by the University of Bath.

Funding declaration: The author received no financial support for the research, authorship and/or publication of this article.

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Article source: Invited. This article was based on a podcast recording, which can be found **here**.

Podcast recorded: Nov 4, 2024; **Revised manuscript received**: Dec 2, 2024;

Publication date: Dec 6, 2024.

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DOI: 10.18609/ioi.2024.015