Institution: University of Oxford

Unit of Assessment: 8 - Chemistry

Title of case study: Mass photometry: enabling next generation research and development of biologics

Period when the underpinning research was undertaken: 2014-18

Details of staff conducting the underpinning research from the submitting unit:

Name(s):	Role(s) (e.g. job title):	Period(s) employed by submitting HEI:
Prof Philipp Kukura	Professor of Chemistry	29/03/2010 – present
Prof Justin Benesch	Professor of Chemistry	01/05/2005 – present
Dr Max Hantke	Postdoctoral Research Assistants	05/06/2017 – 15/09/2018
Dr Gavin Young		01/11/2019 – 31/05/2020
Dr Joanna Andrecka		01/10/2015 – 30/11/2017
Dr Alexander Weigel		01/10/2014 – 31/03/2015

Period when the claimed impact occurred: 2018 – 31st December 2020

Is this case study continued from a case study submitted in 2014? No

1. Summary of the impact (indicative maximum 100 words)

Researchers at the University of Oxford have developed a novel detection technique, known as Mass Photometry, which is capable of weighing molecules with light. It enables the accurate mass measurement of proteins and other molecules in solution, in their native state and without the need for labels – a simple yet powerful capability that is useful across the life sciences, in research and in industry. In June 2018, the University of Oxford spin-out company Refeyn was formed to commercialise the research. Following formation, Refeyn **[text removed for publication]** has since sold and installed **[text removed for publication]** of its first generation Refeyn OneMP mass photometers to pharma companies, research laboratories and facilities worldwide, accelerating and enabling new science for working with and studying biomolecules in both the academic and pharmaceutical space. **[Text removed for publication]**

2. Underpinning research (indicative maximum 500 words)

Professors Philipp Kukura and Justin Benesch from the Department of Chemistry at the University of Oxford have pioneered a new technique known as Mass Photometry (MP). MP relies on the accurate determination of scattering contrast for single molecules as they approach a refractive index interface, for example between a solution and a glass slide. The scattering contrast of biomolecules is closely related to its mass and therefore simply by imaging a molecule as it binds to a microscope slide, the mass can be accurately calculated [**R1**].

This discovery follows a significant period of foundational research. Professor Kukura's group has been working the related technique, interferometric scattering microscopy (iSCAT), for more than 10 years. The group had previously shown the utility of iSCAT for studying molecular motors, such as Myosin 5a [**R2**]. Arguably, the group's most important breakthrough was the 2014 demonstration that iSCAT is sufficiently sensitive to detect and image individual proteins without any labels [**R3**]. Additional advances in detection sensitivity by an order of magnitude [**R4**] led to the further demonstration that light scattering can be used not only to visualise single molecules, which has intrinsic advantages over ensemble-based methods, but also – by careful quantification of the detected signals – to measure their mass with high accuracy, precision, resolution, sensitivity and dynamic range [**R1**].

In recognition of its potential to become an indispensable tool for researchers working across the life sciences, two grants were awarded to further develop the technology and bring it to market: an EPSRC Impact Acceleration Account award (2017) and an ERC Proof of Concept Award (2018).

In Professor Benesch's group, research over the previous decade focussed on developing, enhancing and demonstrating the value of measurements of mass and mass distributions for quantitatively characterising biomolecules and their interactions. In the growing field of native mass spectrometry (MS), his group has led efforts to bring both the experimental approach and its analysis onto a quantitative footing. Advances made by the group have been key in driving mass measurement to the forefront of biophysical investigation, showing that it is the best way to quantify the assembly states of biomolecules and the strengths of their interactions. This work is paving the way to a new era of molecular biology understanding, grounded in the fundamental quantities of physical chemistry [**R5**].

The success and potential of MP comes from the confluence of advances in the Kukura and Benesch groups: the Kukura group's development of a fundamentally new method of measuring mass and the Benesch group's work advancing mass itself as a valuable measure for the life sciences.

3. References to the research (indicative maximum of six references)
All the outputs listed below are journal articles. Oxford staff are highlighted in bold.

R1. Young G, Hundt N, Cole D, Fineberg A, Andrecka J, Tyler A, Olerinyova A, Ansari A, Marklund EG, **Collier MP, Chandler SA, Tkachenko O, Allen J, Crispin M,** Billington N, Takagi Y, Sellers JR, Eichmann C, Selenko P, Frey L, Riek R, **Galpin MR, Struwe WB, Benesch JLP, Kukura P.** Quantitative mass imaging of single biological macromolecules. *Science* **2018** 360:423-327. DOI: 10.1126/science.aar5839

R2. Andrecka J, Ortega-Arroyo JO, Takagi Y, **de Wit G, Fineberg A, MacKinnon L, Young G,** Sellers JR, **Kukura P.** Structural dynamics of myosin 5 during processive motion revealed by interferometric scattering microscopy. *eLife* 2015 4:e05413. DOI: 10.7554/eLife.05413

R3. Ortega Arroyo JO, Andrecka J, Spillane KM, Billington N, Takagi Y, Sellers JR, **Kukura P.** Label-Free, All-Optical Detection, Imaging, and Tracking of a Single Protein. *Nano Lett* **2014** 14:2065–2070. DOI: 10.1021/nl500234t

R4. Cole D, Young G, Weigel A, Sebesta A, Kukura P. Label-free single molecule imaging with numerical aperture-shaped interferometric scattering microscopy. *ACS Photonics* **2017** 4, 211. DOI: 10.1021/acsphotonics.6b00912

R5. Hochberg GKA, Shepherd DA, Marklund EG, Santhanagoplan I, **Degiacomi MT, Laganowsky A, Allison TM,** Basha E, **Marty MT, Galpin MR, Struwe WB, Baldwin AJ,** Vierling E, **Benesch JLP.** Structural principles that enable oligomeric small heat-shock protein paralogs to evolve distinct functions. *Science* **2018** 359 (6378), 930-935. DOI: 10.1126/science.aam7229

Grants evidencing the quality of the research:
EPSRC Impact Acceleration Account award awarded to Kukura (GBP104,000; ref. EP/R511742/1, 2017)
ERC Proof of Concept Award awarded to Kukura (EUR142,050; ref. 813152; 2018-20)

4. Details of the impact (indicative maximum 750 words)

Pathway to impact: from research to commercialisation

The research and development of mass photometry (MP) in Kukura and Benesch's research groups attracted attention after publication in Science in 2018 [**R1, E1-2**]. The combination of experimental power and feasibility led many academic institutions and commercial enterprises to express an interest in obtaining the technology to analyse their own samples. In the 18 months following publication of MP, an enormous variety of samples and scientific questions were brought to the Kukura lab by more than 30 international collaborators from a variety of scientific backgrounds. The interests of these groups ranged from developing and interrogating antibody-based pharmaceuticals to the manufacture and quality control of inorganic nanoparticles.

Throughout MP's development, the researchers worked closely with Oxford University Innovation (OUI), the technology transfer company for the University of Oxford, to protect the new IP being developed. Recognising the potential impact of this technology, OUI filed 2 patents, with one covering the key aspects of the MP device, *Interferometric scattering microscopy* [**E3**], and another a key implementation of the technology, *Method of determining lipoprotein concentration in solution using light scattering* [**E4**]. The former has been granted in the US and in Europe.

OUI and the academic team identified the formation of a spin-out company as the most appropriate route to maximise the impact from this platform technology. In June 2018, University of Oxford spin-out company Refeyn (initially known as Arago Biosciences) successfully raised **[text removed for publication]** from several sources, including Oxford Sciences Innovation, to further develop the technology and make it widely available as an instrument accessible to non-expert users. Within months, the company had made its first sales to academic institutions and continued to expand the capabilities of the microscope. The founding of the company was extremely rapid: an EPSRC IAA award in October 2017 enabled construction of a first commercial prototype, which was presented at an international conference in February 2018, 2 months before the original manuscript was published, which in turn was 4 months before Refeyn was founded. [**E5**]

Following a highly successful first 8 months in operation, Refeyn raised a further **[text removed for publication]** led by Foresight Williams and Oxford Sciences Innovation with the aim of expanding its development pipeline and scaling up production of its existing product, the Refeyn One^{MP}. Subsequently, Refeyn raised another **[text removed for publication]** during the COVID-19 pandemic, led by high profile US investors Northpond Ventures [**E5**].

Innovative new technology

The ability to observe the often-subtle interactions between proteins is crucial for studying the processes that underpin life itself. Only through a deep understanding of these processes can we begin to develop the next generation of biopharmaceuticals to tackle previously untreatable diseases. The MP approach 'weighs molecules with light', providing immediate insight into the state of proteins and other molecules.

In mass photometry, the light scattered by a molecule attached to the measurement interface interferes with light reflected at that interface. The interference scales linearly with object mass. The Refeyn One^{MP} detects this scattered light, using the signal to count molecules and measure their mass. This is possible in a wide range of native buffer solutions, without the need for labels.

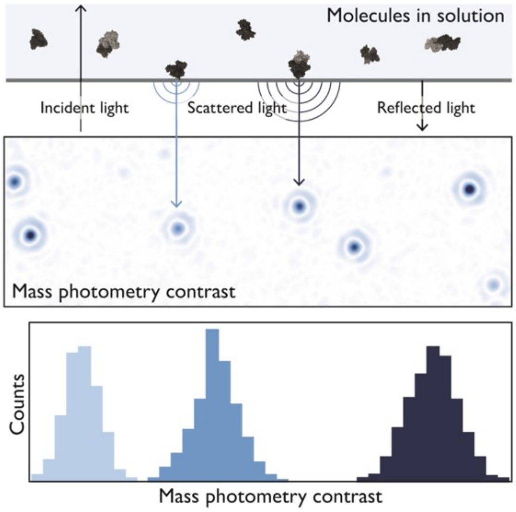

Figure 1. Refeyn One[MP] benchtop mass photometry device (above).
Figure 2. The principle of mass photometry (right).

The Refeyn One[MP] featured in The Scientist's (a professional magazine for life scientists) Top 10 Innovations of 2019. The Boris Magasanik Professor of Biology at the Massachusetts Institute of Technology **[text removed for publication]** was quoted as saying "*The most valuable thing with this instrument is that we can look at complex proteins-macromolecular complexes and figure out what are the components in that mixture, and do we detect any issue with stability. It's a very time-consuming process in typical workflow*." [**E6**]

In 2019 Refeyn went on to win the Royal Society of Chemistry's Emerging Technologies Competition Award and an R&D 100 award in the analytical/test category [**E11**].

Commercial sales and use

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The instruments are being used across vaccine development, cell and gene therapy production, Quality Assurance/Quality Control of biologics, and next generation drug discovery [**E5**]. More than 20 academic publications have explicitly used the instrument in the broader and more specific bioanalytical context [**E7**]. For example, AstraZeneca have used Refeyn One[MP] in COVID-19 research to study the spike protein of the SARS-CoV-2 virus and its interaction with the ACE2 receptor [**E8**], and for drug discovery to explore the characterization of star polymers for future biophysical targeting of tumours [**E9**]. The National Heart, Lung and Blood Institute of the National Institutes of Health (USA) used MP to measure the affinity of protein-protein interactions on a single-molecule level, concluding '*The quality and information content of the MP data, combined with simple and fast measurements and low sample consumption makes MP a new preferred method for measuring strong protein-protein interactions*.' [**E10**]

In just 2 years the uptake and variety of uses seen across the scientific community show that mass photometry is a transformative technology enabling a new way to analyze molecules. It has opened up new possibilities for bioanalytics and research into the functions of biomolecules across the life sciences.

5. Sources to corroborate the impact (indicative maximum of 10 references)

E1. Article in Science Translational Medicine – 'A New Method to Weigh Biomolecules', In The Pipeline blog, Derek Lowe, published 10 May 2018, corroborating the early interest in MP
E2. Article in Chemical and Engineering News – 'Scattered light weighs single biomolecules', Mark Peplow, 2018, Vol 96, Issue 18, corroborating the early interest in MP
E3. Patent: P. Kukura, A Weigel, WO2018011591A1, *Interferometric scattering microscopy*; part of the key IP on which Refeyn was founded

E4. Patent application: J. Andrecka, G. Young, P. Kukura, PCT/GB2018/053508, *Method of determining lipoprotein concentration in solution using light scattering*; part of the key IP on which Refeyn was founded
E5. Letter of support from Chief Executive Officer of Refeyn (Jan 2021) corroborating details relating to the company, its revenue and employees, and testimonials from key customers
E6. The Scientist magazine's report on their Top 10 Innovations of 2019 (published 1 Dec 2019), corroborating their selection of the Refeyn One^{MP} and including the testimonial from the Boris Magasanik Professor of Biology at the Massachusetts Institute of Technology
E7. Refeyn website: List of academic publications using the Refeyn One^{MP}
E8. Application note from the Refeyn website corroborating AstraZeneca and Refeyn's use of Refeyn One^{MP} in the study of COVID-19
E9. Journal article: R. England et al, Synthesis and Characterization of Dendrimer-Based Polysarcosine Star Polymers: Well-Defined, Versatile Platforms Designed for Drug-Delivery Applications, Biomacromolecules, 2020, 21, 8, 3332–3341, corroborating use of the Refeyn One^{MP}
E10. Journal article: D, Wu & G Piszczek, Measuring the affinity of protein-protein interactions on a single-molecule level by mass photometry, Analytical Biochemistry, 2020, 592 113575, corroborating use of the Refeyn One^{MP}
E11. Awards: Royal Society of Chemistry Emerging Technologies Competition (2019) and R&D 100 Awards (2019)