Institution: University of Oxford		
Unit of Assessment: 9: Physics		
Title of case study: The Nanoimager - from a physics lab to COVID-19 testing via spin-out success		
Period when the underpinning research was undertaken: 2006 – 2020		
Details of staff conducting the underpinning research from the submitting unit:		
Name(s):	**Role(s) (e.g. job title):**	**Period(s) employed:**
Achillefs Kapanidis	Professor of Biological Physics	2005 – Present
Nicole Robb	Research Fellow and PDRA	2011 – 2020
Period when the claimed impact occurred: May 2016 – December 2020		
Is this case study continued from a case study submitted in 2014? N		

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

Kapanidis developed and commercialised a unique fluorescence microscope for single-molecule imaging, the Nanoimager. Although other microscopes of this type exist, the Nanoimager is significantly smaller, much simpler to operate, and around 30-50% of the cost. The microscope and associated software reveal the distribution, structures and motions of biomolecules and nanoparticles on surfaces, in solution and in living cells, accelerating academic and industrial research, diagnostics, drug discovery, chemical analysis, and education. Custom software, services and assays for the detection of specific target entities have been developed for several applications in academia and industry. For example, detection of COVID-19 in clinical samples, substantially faster than existing rapid diagnostic tests, is being developed commercially. The Nanoimager was commercialised via the spinout Oxford Nanoimaging (ONI) in 2016. ONI has rapidly grown to 98 employees, expanded to the USA and has sold microscopes worldwide exceeding GBP5,000,000 annually.

2. Underpinning research (indicative maximum 500 words)

Invention of Nanoimager (2005 – 2016)

The invention of single-molecule fluorescence methods revolutionized biophysics and molecular biology by breaking the diffraction limit in microscopy, offering real-time views of biochemical reactions and enabling ultrasensitive detection. When Kapanidis joined Oxford (2005) he began pursuing miniaturised single-molecule imaging (first grant in 2006): the initial goal was to produce desktop devices that would replace the unstable and cumbersome microscopes, occupying entire rooms and requiring laser interlocks and massive optical tables, that were the only devices then available. In 2009, Kapanidis refocused on wide-field microscopy **[1]** to offer higher throughput and biological context for localisation-based super-resolution microscopy **[2, 3].** Initial work on miniaturised microscopy was pursued with the company Chelsea Technologies Group. In 2011, the Kapanidis group completed a proof-of-concept bench-top wide-field microscope; in 2013, Kapanidis and his student Bo Jing came up with several innovations and a radically new design, the first successful 'Nanoimager' prototype and associated software **[4]**.

Antimicrobial Resistance (AMR)

The Nanoimager device has been used to carry out single-molecule analysis of double-stranded DNA in living bacteria to improve understanding of how double-strand breakages are repaired. This is crucial to understand how bacteria can become resistant to clinically relevant antibiotics that produce DNA breakages. This research had the objectives of identifying the mode of action of existing and potential new antimicrobial lead compounds that target chromosomes and DNA-binding proteins in bacteria, and of using the Nanoimager to develop a rapid antimicrobial-susceptibility test to evaluate the response of bacteria from individual patients to antibiotic treatments; faster tests allow more rapid and informed decisions on patient treatment and help avoid the use of broad-spectrum antibiotics treatments that exacerbate antibiotic resistance. The technique developed uses bright-field and ultra-sensitive fluorescence microscopy to examine the observable properties (phenotypes) of a single cell in the absence and presence of antibiotics and determine whether bacteria are antibiotic-resistant or susceptible. This information is combined with bacterial identification and detection of gene expression associated with antimicrobial resistance through highly multiplexed single-molecule fluorescence in-situ

hybridization (a method for detecting and localizing specific DNA and RNA sequences). These techniques, together with the Nanoimager, were then used for research on the influenza virus, and more recently on SARS-CoV-2, the virus that causes COVID-19.

Influenza and COVID-19 Virus Testing

Kapanidis and Robb discovered a novel, calcium-mediated interaction by which the surface of viruses can be enveloped with DNA and labelled by fluorophores attached to the DNA **[5]**. This enabled rapid (in one minute) and sensitive labelling and detection of influenza and other viruses using single-particle tracking and particle-size determination (patent GB1817802.0). Work to combine this non-specific optical assay with virus-specific labelling approaches (aptamers, hybridization probes and antibodies) was then applied to the emerging SARS-CoV-2 at the beginning of 2020 as the outbreak grew to a pandemic. A team including Robb and Kapanidis and their student Shiaelis worked with collaborators at the John Radcliffe Hospital, Oxford to develop a methodology using the Nanoimager for virus identification via a convolutional neural network; after the network is trained on large sets of images of single particles of different viruses, the network can identify the presence of a specific virus in biological and clinical samples by being able to identify the presence of specific viral particles in sets of images from these samples. The assay (patent GB2006144.6) achieves labelling, imaging and virus identification in less than five minutes; the trained neural network is able to differentiate SARS-CoV-2 from negative clinical samples, as well as from other common respiratory pathogens such as influenza and seasonal human coronaviruses, with accuracies ranging from 70-95% per individual particle, leading to high confidence (>99%) in identifying virus-positive samples with significant viral load **[6]**.

3. References to the research (maximum of six references)

1. Holden SJ, Uphoff S, Hohlbein J, Yadin D, Le Reste L, Britton OJ, Kapanidis AN. Defining the limits of single-molecule FRET resolution in TIRF microscopy. Biophysical Journal, 2010, 99, 3102-11. https://doi.org/10.1016/j.bpj.2010.09.005 (journal article)

2. Holden SJ, Uphoff S, Kapanidis AN. DAOSTORM: an algorithm for high-density super-resolution microscopy, Nature Methods, 2011, 8, 279-80. https://doi.org/10.1038/nmeth0411-279 (journal article)

3. Uphoff S, Reyes-Lamothe R, Garza de Leon F, Sherratt D, Kapanidis AN. Single-molecule DNA repair in live bacteria. PNAS, 2013, 110, 8063-8. https://doi.org/10.1073/pnas.1301804110 (journal article)

4. UK priority application, entitled "Compact microscope", filing number GB1318919.6, October 2013 (25/10/13); PCT application, entitled "Compact microscope", filing number PCT/IB2014/065639, October 2014 (27/10/14). (patent application)

5. Robb N, Taylor JM, Kent A, Pambos OJ, Gilboa B, Kapanidis AN. Rapid functionalisation and detection of viruses via a novel Ca^{2+}-mediated virus-DNA interaction. Scientific Reports, 2019, 9, 16219. https://doi.org/10.1038/s41598-019-52759-5 (journal article)

6. Shiaelis, N, Robb N, Kapanidis AN et al. Virus detection and identification in minutes using single-particle imaging and deep learning. medRxiv preprint (2020) https://doi.org/10.1101/2020.10.13.20212035 (preprint article)

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

Oxford Nanoimaging

Between 2005 and 2015, large microscope companies modified their products to offer commercial microscopes to the growing numbers of biologists who wanted access to super-resolution imaging (a technique recognized by the 2014 Nobel Prize in Chemistry). Such microscopes allowed scientists in academia and industry to study individual molecules, even inside living cells, and distinguish structures as small as 10 to 20nm – less than one fifth the size of a flu virus. However, these instruments are largely inaccessible to most researchers in

academia and industry due to their high cost (GBP250,000 to GBP500,000), complexity and the infrastructure and know-how required to house and operate them. Further, apart from super-resolution imaging, the commercial instruments do not offer many options for single-molecule detection, thus severely limiting the application of single-molecule methods in biosensing and drug discovery, and preventing industrial scientists from exploiting the high sensitivity of these methods as well as their ability to monitor directly molecular interactions and conformational changes. The industrial and scientific community needed compact, robust and affordable microscopes and software, effective in any environment e.g., biochemistry labs, hospitals, and quality-control industrial labs. To meet these needs, Oxford Nanoimaging (ONI) was founded by Kapanidis and Jing in May 2016 to manufacture and sell the Nanoimager. The design featured two modules (each with a footprint of less than an A4 page) linked with optical fibres: the excitation module included three lasers in the visible wavelength range; the microscopy module included most of the optics for sample excitation as well as for the detection of the fluorescence emission. Crucially, the microscopy module featured a single aluminium plate (size only: 20cm x 22.5cm x 1.2cm) that acted as a miniaturised optical table, to which all optics and the sCMOS camera were attached, utilising both top and bottom surfaces. The integrated construction, along with the very small size of the microscope and the short distance of the optical paths from the surface of the motherboard (1 to 3cm), minimised the relative motion between optical components. This, together with a vibration-absorbing gel (linking the motherboard to the microscope module housing), provided outstanding vibration isolation and very low thermal drift in all three dimensions.

The first prototype also featured a novel and inexpensive way to achieve autofocus, involving a 780nm laser beam coupled to a multi-mode fibre to produce a focused spot at the glass/sample interface. When imaged on the sCMOS camera, any axial motion of the sample was identified as a widening of the scattered image of the beam. This could be rectified after comparison with a set of calibration images by sample motion using a piezo stage. Combined with close temperature control this gives extraordinary stability, showing lateral and axial drifts that greatly outperform other microscopes. In 2015, the team developed the second prototype, which combined outstanding spatial-temporal resolution, even better stability (by means of a novel drift-cancelling geometry for the sample stage, further minimising any remaining thermal drift and achieving very low lateral and axial drifts (which are 30nm $hr^{-1}K^{-1}$ and less than 1nm $hr^{-1}K^{-1}$, respectively), while maintaining the small size (a shoebox-size microscope module and a desktop-tower module for lasers/electronics). This version of the Nanoimager was also able to localise individual molecules with a resolution of 20nm, track single molecules and vesicles inside cells and in free solution, and enabled use of single-molecule fluorescence-detected resonance energy transfer (smFRET) for the characterisation of specific molecular interactions and intra-molecular dynamics. Since the Nanoimager is laser-safe, it is also ideal for outreach and teaching.

Jing joined ONI as Chief Technology Officer and is now CEO; Kapanidis became a board director and consultant. Since its formation in 2016, ONI has attracted GBP22,400,000 **[A]** in investment, had 98 employees (headcount: 98) in 2019 (up from 60 in 2018) **[B]** and achieved a turnover of GBP5,115,000 in 2019 (up from GBP2,832,000 in 2018) **[B]**. This rapid growth has seen expansion to the USA with a base in California. The Nanoimager still uses many UK parts, stimulating the local economy. ONI has recorded numerous microscope sales worldwide (UK, US, Italy, Netherlands, Israel, Japan, Mexico, Australia etc.) and many early adopters purchased additional units. In recognition of this success, ONI won the “Start-up of the Year” award of the Institute of Physics **[C]** and was chosen as a Fast-Track-100 company by the Sunday Times **[D]**, both in 2018. Kapanidis and Jing also won the BBSRC “Innovator of the Year” award in 2019 in both the commercial and overall winner categories **[E]**.

The "table top" Nanoimager, first commercialised in 2016 by Oxford Physics spin-out, Oxford Nanoimaging (ONI).

Wide Range of Users

The Nanoimager offers a wide range of single-molecule fluorescence capabilities (single-molecule localization and tracking, super-resolution imaging, single-molecule fluorescence resonance energy transfer (smFRET), single-cell fluorescence), and has served as a platform for further development of microscope hardware by ONI, including structured illumination microscopy and microfluidics. Nanoimagers are integrated with dedicated software, services and assays for several applications in academia and industry, some of which are detailed below. Medical diagnostics and drug discovery have been immediate beneficiaries, with users in universities, medical schools, and national research institutes. For example, the Head of IMAXT laboratory at Cancer Research UK, who uses the Nanoimager to help build 3D versions of breast tumours to improve tumour diagnosis and treatment, said "*We really hope that what we are doing is going to be the foundation of new discoveries, a new understanding of better ways to diagnose and treat tumours*" **[F]**. An oncologist at the Sheffield Medical School commented that: *"The Nanoimager brings single molecule measurements out of the dark room and into any research lab. The Nanoimager is stable, sensitive and affordable allowing researchers to quickly get to grips with performing their measurements"* **[G]**. The Nanoimager has also been adopted for microbiology work, as it is compatible with constrained environments such as Biosafety-level II/III/IV spaces in clinical microbiology labs and public-health institutes: e.g. from a scientist at the MRC Centre for Molecular Bacteriology and Infection where non-pathogenic and pathogenic *E. coli* are studied: "*Single molecule microscopy is in fact a game changer for us. The Nanoimager is extremely user-friendly"* **[H]**.

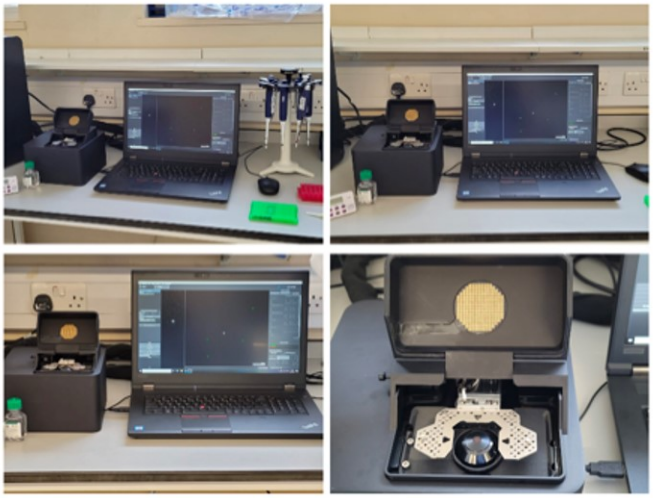
The Nanoimager has been attractive for industry, including pharmaceutical companies working on drug-discovery platforms and antibiotic development, DNA-sequencing companies that require nanoscale characterisation of their substrates, and biotech companies pursuing disease biomarkers. Evox Therapeutics Ltd are creating novel therapeutics for the treatment of rare and severe diseases; the Co-Founder and Chief Operating Officer commented: *"Evox Therapeutics Ltd, an Oxford-based exosome therapeutics company, has used the Nanoimager extensively over the last couple of years. As a company carrying out exosome-focused research and development the Nanoimager forms an integral part of our analytical suite of instrumentation and it plays an important role in studying and characterising, for instance, exosome internalisation and trafficking in target cells and drug loading into exosomes, and in the development of extended product characterisation criteria for future regulatory submissions. The use of the Nanoimager has furthered our understanding of exosome biology and the application of exosomes as therapeutics and the images it produces often feature in our presentations and posters, helping to simplify, visualise and communicate the potential and power of exosome biology"* **[I]**. Furthermore, Astra Zeneca has collaborated with ONI to develop single-molecule assays for drug discovery. "*We use single-molecule fluorescence to study the interactions of our protein targets with other proteins and small molecules...The NanoImager allows us to easily monitor ternary complex formation using purified proteins via immobilisation of one of the partner proteins to a chemically modified surface. The single-molecule method requires very small amounts of sample and contributes to our understanding of the mechanism of action of our molecules… it is a very impactful tool to accelerate a drug discovery process*", commented the Vice President, Global Head of Structural Biology, Biophysics and Fragment-Based Lead Generation at Astra Zeneca **[J]**.

COVID-19 Testing

Kapanidis and Robb led a multidisciplinary programme to develop an assay that achieved labelling, imaging (using the Nanoimager) and virus identification in less than 5 minutes without requiring any lysis, purification or amplification steps. *"The collaboration between Achilles Kapanidis' "Gene Machines" laboratory and ourselves (Derrick Crook, Nicole Stoesser) in the Modernising Medical Microbiology consortium/Oxford University Hospitals NHS Foundation Trust came about through a proof-of-principle project to develop a single-cell nanoimaging approach to rapid bacterial pathogen diagnostics and phenotyping... The onset of the pandemic led to a new opportunity to deploy the Nanoimager in the John Radcliffe Hospital in order to be used for proof-of-principle work in developing a new rapid diagnostic approach to diagnosing SARS-CoV-2 in respiratory samples, using a method developed by Nicole Robb and Nicolas Shiaelis"* **[K].** This assay was the founding Intellectual Property for the formation of separate new spin-out company, Oxford Rapid Diagnostic Technologies (ORDTech), to develop and commercialise the assay technology **[L]**.

Separately from Kapanidis and Robb and independently of Oxford University, ONI has been funded by Innovate UK to work with the Medicines Discovery Catapult and Imperial College London's Healthcare NHS Trust on testing and deployment of their own new single-step SARS-CoV-2 detection assay, which will allow direct swab testing by placing a nasal/pharyngeal sample into a collection tube containing a viral lysis/labelling buffer. This assay differs from that used by Kapanidis and Robb but also runs on the Nanoimager with results in under 10 minutes and has the potential to boost national testing capacity **[M]**.

Nanoimager in use in the Microbiology Lab, John Radcliffe Hospital, Oxford



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

[A] Crunchbase website information on Oxford Nanoimaging investment: https://www.crunchbase.com/organization/oni/company_financials
[B] Companies House ONI Annual Report 2019
[C] IoP Press Release announcing ONI as "Start-up of the Year"
[D] Sunday Times article announcing ONI as a Fast-Track-100 company
[E] BBSRC press release announcing winners of BBSRC "Innovator of the Year" award
[F] Quote on ONI website from Head of IMAXT laboratory at Cancer Research UK
[G] Quote on ONI website from Oncologist at Sheffield Medical School
[H] Quote on ONI website from MRC Centre for Molecular Bacteriology and Infection, London
[I] Email from Co-Founder and Chief Operating Office, Evox Therapeutics
[J] Email and quote on ONI website from Vice President, Global Head of Structure, Biophysics & Fragment-Based lead Generation at AstraZeneca
[K] Email from Modernising Medical Microbiology consortium/ NHS Foundation Trust
[L] Reuters website article: https://www.reuters.com/article/us-health-coronavirus-britain-antigen-idUKKBN27014F
[M] Description of Innovate UK Funded project ONI-CoV2RNA on the UKRI website