Research Assessment Exercise 2020

Impact Case Study

University: The Hong Kong University of Science and Technology
Unit of Assessment (UoA): 7 Physics and Astronomy
Title of case study: Advanced Optical Microscopies for Biomedical Imaging

(1) Summary of the impact

Laser physics and optics research by Prof Shengwang Du and his group has created a new generation of super-resolution and live cell bioimaging technologies now being used to address major challenges in biomedical science, among other uses. The group's breakthroughs in providing clear and detailed images of cells needed for biomedical research, and other materials, have achieved a) impact for research and research practitioners in and beyond Hong Kong; and b) economic impact, through two start-up companies: NanoBioImaging (NBI) Ltd and Light Innovation Technology (LiT) Ltd. NBI, founded in December 2013, commercialized the group's SRiS super-resolution optical imaging systems. LiT, founded in June 2016, develops and manufactures Line Bessel Sheet (LBS) light-sheet microscopes and provides biomedical imaging and material topography solutions, with about 40 groups internationally now using its imaging services. The two companies have delivered their cutting-edge equipment to research institutes and industry in Hong Kong, Macau, mainland China, and Taiwan.

(2) Underpinning research

Starting from 2012, Du's group invented and developed three key bioimaging technologies: i) super-resolution localization optical imaging; ii) LBS light-sheet microscopy; and iii) dual-beam shear differential interference contrast (DInC) microscopy. The group collaborated with HKUST researchers from life science, chemistry, biological engineering, and mechanical engineering, who provided valuable testing platforms for the technologies and were the first users, advancing their research by providing important and novel practical solutions for investigating tiny structures in cells and tissues in humans, animals and plants, as well as nanomechanical structures. The new capabilities in microscopy have facilitated, for example, leading research into neurological diseases such as dementia, its causes, and progression at sub-cellular level, and potential treatments, among other uses.

I. Super-Resolution Localization Fluorescence Optical Imaging

While research establishing the scientific principles of super-resolution microscopy received the Nobel Prize in Chemistry in 2014, implementing the principles in a commercially viable product has been a major challenge. Prior to 2015, commercial manufacturers such as Nikon, Zeiss and Leica faced difficulties in making their products user-friendly, particularly in stability, sample drafting, and multicolor channel cross-talk. By the end of 2014, the Du group had overcome these limitations, by developing a user-friendly two-color super-resolution microscope through the invention of three key technologies: i) an active locking system to solve the sample drafting problem; ii) simultaneous imaging of two-color channels without cross-talk; and iii) an optimized imaging buffer for balanced switching characteristics for both dyes. Together, this allowed the technology to be used beyond traditional, highly controlled environments, and enabled life scientists to distinguish between the tiniest molecules and cellular components, with the microscope built to achieve <20 nm XY resolution and <50 nm Z resolution.

As the technology was being developed and its commercial potential recognized, Du and business partners Prof Jianping Wang and Prof Chun Xue (computer scientists from City University of Hong Kong) formed NBI in December 2013. Du's group successfully transferred the technology through HKUST's Technology Transfer Center. A technical research paper was published in 2015 [**See Section 3, R1**], and two related China patents were granted in 2017 [CN 104515760 B] and 2018 [CN 104458683 B]. In collaboration with life scientists and chemists, the new imaging tool was fully tested and used to study a wide range of biological and chemical problems, such as neuronal

activity-dependant synapse development – the mechanism by which brains turns on and off neuronal activities; and the precise biochemical mechanisms involved in synaptic transmission [**R2**, **R3**].

II. LBS Light-Sheet and Dual-Beam Shear DInC Microscopies

Since the 1980s, scanning confocal microscopy (SCM) has been the most popular form of optical microscopy for life scientists because it can resolve 3D cell structures. However, biological samples under SCM suffer serious phototoxicity, making it unsuitable for long-time live cell imaging in three dimensions. However, such imaging is essential for life scientists engaged in cell signaling and trafficking research to understand neurodegenerative diseases and identify potential treatments and cures, among other applications.

Recently developed light-sheet microscopy (LSM) appeared to provide a solution to this problem: 3D capability with much lower phototoxicity (1/10,000 – 1/1,000 of a typical SCM). However, the optical diffraction prevents a Gaussian beam-based LSM from producing ultrathin light sheets and a large view area simultaneously. In 2014, Eric Betzig made a breakthrough by inventing the lattice light-sheet microscope with non-diffracting ultra-thin light sheets. However, in 2015, Du's group showed that lattice geometry is not necessary for generating non-diffracting light sheets, going on to invent their own non-diffracting multicolor light-sheet microscope implemented with a Line Bessel Sheet (LBS) **[R4] [R5]**, and applied for an international patent [PCT/CN2016/073199 (filed 02/02/2016)].

In June 2016, Du, his two PhD students Teng Zhao and Luwei Zhao, and Prof Michael Loy (HKUST Physics Professor Emeritus) started LiT Ltd to transfer the technology through the University's Technology Transfer Center and commercialize the LBS light-sheet microscopy and live-cell imaging technologies. In 2019, with an LiT-supported research contract, Du developed a dual-beam shear DInC microscope for material topography, which extends LiT's scope to material engineering and applications **[R6].** A patent application was filed on 23/04/2018 [PCT/CN2018/ 080104].

(3) References to the research

[R1] Zhao,T; Wang, Y; Zhai, YL; Qu, XX; Cheng, AF; Du, SW; and Loy, MM. 2015. "A user-friendly two-color super-resolution localization microscope", Optics Express **23**, 1879.

[R2] Huang, HQ; Lin, XC; Liang, ZY; Zhao, T; Du, SW.; Loy, MM; Lai, KO; Fu, AK; and Ip, NY. 2017. "Cdk5-dependent phosphorylation of liprinα1 mediates neuronal activity-dependent synapse development", Proceedings of the National Academy of Sciences of the United States of America (PNAS) **114**, E6992.

[R3] Cheng, AF; Zhao, T; Tse, KH; Chow, HM; Cui, Y; Jiang, LW; Du, SW; Loy, MM; and Herrup, K. 2018. "ATM and ATR play complementary roles in the behavior of excitatory and inhibitory vesicle populations", PNAS, **115**, E292.

[R4] Zhao, T; Lau SC; Wang, Y; Su, YM; Wang, H; Cheng, AF; Herrup, K; Ip, NY; Du, SW; and Loy, MM. 2016. "Multicolor 4D fluorescence microscopy using ultrathin Bessel light sheets," Scientific Reports **6**, 26159.

[R5] Du, SW; Zhao, T; and Zhao, LW. 2019. "Light sheets with extended length", Optics Communications. **450**, 166.

[R6] Zeng, ZH; Zhang, CB; Du, SW; and Chen, X. 2019. "Quantitative surface topography of martensitic microstructure by differential interference contrast microscopy", Journal of the Mechanics and Physics of Solids **124**, 102.

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(4) Details of the impact

The Du group's research is achieving significant impact on scientific research and its practitioners by providing cutting-edge microscopy technology needed to progress investigations that address critical issues in biomedical research, such as the causes and potential cures for neurodegenerative diseases. Through successful commercialization of the resulting products, it is also achieving economic impact reflected in sales and jobs created by the HKUST start-up companies NBI and LiT, co-founded by Prof Du and physics postgraduate students [see **Section 5, S1** and **S2]**, as well as wider strategic economic impact for Hong Kong in helping achieve policy implemented over the past decade to develop the city as a center for research and innovative technology.

NanoBioImaging (NBI) Ltd paved the way for the impact of Du and his group's work, being established in December 2013 with private funding of HK$4 million. The company, initially based at Hong Kong Science Park, has achieved economic impact evidenced by total sales income of HK$4.8m up to January 2019 and delivered three SRiS super-resolution imaging systems to biological and medical research labs at University of Hong Kong (HKU), Sun Yat-Sen University in Guangzhou and Academia Sinica in Taiwan **[S3]**. An example of impact on scientific research and research practitioners is the imaging system's use to identify a distinct pathway that mediates cell wall formation during cell expansion and division in research led from Chinese University of Hong Kong that has shed important new light on plant growth and development **[S4]**. The company has gone on to recruit nine full-time employees. Significantly, in 2018, NBI received fresh investment of HK$10 million from the start-up platform and fund, Hong Kong X-Tech, having been identified by Hong Kong-X as one of six "stand-out" and "rising Hong Kong technology companies" **[S5]**. Neil Shem, Chairman of Hong Kong X, said at the ceremony attended by the Secretary for Innovation and Technology of the HKSAR to announce the investments: "The performance of the six portfolio companies selected for the accelerator program is encouraging and proves the significant potential of Hong Kong-based technology start-ups" – evidence of the company's contribution to realizing Hong Kong's policy to diversify its economy by becoming a center for research and technological innovation.

Meanwhile, **Light Innovation Technology (LiT) Ltd** was co-founded by HKUST physics professors (Du and Loy) and PhD students (Teng Zhao and Luwei Zhao) in June 2016 with HK$6 million angel investment to commercialize their LBS light-sheet microscopy for 5D live-cell biomedical imaging, successfully providing enhanced imaging capabilities to scientists. LiT has grown to 11 full-time and part-time employees in Hong Kong and mainland offices, indicative of its economic impact. By March 2019, it had achieved total sales income of HK$7.8 million, from five microscope systems sold to biomedical industry and research labs at Shanghai Zhongqiao Xinzhou Biotechnology Co Ltd; HKU; University of Macau; and HKUST **[S6]**. It has also provided imaging services to more than 40 groups in mainland China, Hong Kong, Macau, Taiwan, and the US to facilitate their biomedical research.

Dan Yang, Chair Professor of Chemistry at HKU, affirmed the significance and value of the LiTone LBS microscope that her university purchased and now uses for research she leads on natural product synthesis, including the development of fluorescent markers and sensors to label specific structures and substances involved in metabolism inside living cells. She noted it had proven to be a "*perfect instrument*"… "*as it allows us to observe the exact distribution of our markers and sensors in 3D with improved sharpness compared to the old technology like confocal microscopes. Most importantly, the LiTone LBS is a better tool when live cell imaging is needed, as it offers much reduced phototoxicity during imaging, which is critical to our experiments related to oxidation chemistry where reactive oxygen species (ROS) will be produced when the sample undergoes strong illumination exposure."* **[S7]** The products being investigated may have important anti-inflammatory, anti-bacterial and anti-tumor uses.

Researchers at Shanghai Zhongqiao have been similarly impressed. In testimony, General Manager Wan Wu wrote: "*As a biotech company with missions to develop cell products for experimental use and to study and develop cell therapeutic strategies, we need to observe the 3D structure inside the cells at high resolution while the cells are alive and healthy for a long time. The*

old technology we have been using, such as conventional fluorescent microscopes or confocal microscopes, are either unable to perform 3D imaging, or have enough scan speed to record the vital process we need to observe, and in most cases they tend to kill the cells during observation because the cells are exposed under strong light exposure. We have confirmed that the LiTone LBS has the unique ability in capturing the high-resolution movies of 3D intercellular structure at 1,000 times faster than the confocal microscopes. Meanwhile, [the] LBS use[s] very little light for illumination so that the observation can carry on long enough for our purposes." **[S8]**

The significance of this breakthrough technology for scientific research has caught public attention in Hong Kong and mainland China, reflected in extensive media coverage [**e.g. S9-10]**.

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

[S1] LiT: https://www.lit.com.hk [LiT company data on file]
[S2] NBI: http://www.nanobioimaging.com [NBI company data on file]
[S3] Letter, Yanbing Jiang, Chairman and CEO, NanoBioImaging Ltd [on file]
[S4] Wang, H. et al, Zhuang, X and Wang, X, et al. 2016. A Distinct Pathway for Polar Exocytosis in Plant Cell Wall Formation**.** *Plant Physiology*, 172(2): 1003–1018. http://www.plantphysiol.org/content/172/2/1003
[S5] Hong Kong X announcement: http://www.hkxtech.com/hong-kong-x-technology-fund-investment/?lang=en
[S6] Letter, Teng Zhao, CEO, Light Innovation Technology (LiT) Ltd [on file]
[S7] Letter, Dan Yang, Chair Professor of Chemistry, University of Hong Kong [on file]
[**S8]** Letter, Wan Wu, General Manager, Shanghai Zhongqiao Xinzhou BioTech Co Ltd [on file]
[S9] 浙江省醫學科學院使用 LiT 激光片層顯微鏡發現幹細胞誘導心肌細胞運動 http://www.zjams.com.cn/20180719/2018071900002.htm
[S10] 文匯報：http://paper.wenweipo.com/2017/08/18/ED1708180001.htm