



# Scottish Microscopy Society 49<sup>th</sup> Annual Symposium





School of Life Sciences University of Dundee 7<sup>th</sup> November 2023



 $09{:}15-09{:}50$  Registration with tea/coffee; Trade Exhibition and poster hanging

	Session 1. Chair: Dr Charlotte Buckley
09:50 -	Charlotte Buckley, Scottish Microscopy Society
10:00	Chairperson, University of Strathclyde
	Welcome!
10:00 -	JEOL-SPONSERED KEYNOTE TALK
10:45	Dr Laura Young, University of Newcastle
	Imaging cells in the living human eye with
	adaptive optics
10:45 –	Invited Short Talk
11:00	Professor Carlos Penedo, University of St Andrews
	The lumicks c-trap microscope: a new facility for high
	resolution mechanobiology
11:00 –	Dr Cristina Martinez-Gonzalez, University of Edinburgh
11:15	Whole-brain light sheet imaging of behaviourally-
	activated neurons
11:15 -	Technobite
11:20	Laser2000
au	
	Morning Tea/Coffee & Biscuits; Trade
	Coosien O. Cheir Dr Alex Pressett
44.50	Session 2: Chair Dr Alan Prescott
11:50 -	Freya whiteford, University of Glasgow
12:05	The OpenFlexure Microscope: Smart microscopy for
40.0E	everyone with open source hardware
12:05 -	Invited Short Talk
12:20	Dr Ramasubramanian Sundaramoortny, University of
	Dundee
	the people of the research community
12.20 -	Megan Clapperton, University of Strathclyde
12.20 -	Searching for biomarkers of tonsillar disease
12:35 -	Invited Short Talk
12:50	Dr Callum Graham, Historic Environment Scotland

12:50 -	Technobite
12:55	JEOL
12:55 –	Tecnobite
13:00	Miltenyi Biotec

Lunch Break, Trade Exhibition, Posters

14:00 - 14:30

Expansion Microscopy Technique Discussion in Breakout Room Chair: Dr Nikki Paul, Room: WTB SR

Session 3: Chair Dr Tom Vettenburg		
14:30 – 14:45	Scottish Microscopist of the Year Winner Dr Liam Rooney, University of Strathclyde	
14:45 – 15:00	Giedrė Astrauskaitė, University of Glasgow Remote focusing for 2-photon microscopy to follow action potential propagation transmurally in acute rabbit heart slices	
15:00 – 15:15	Prof Paul Campbell, University of Dundee Ultrafast Microphotography at sub-microsecond framing rates	
15:15 – 15:30	Rebecca Craig, University of Strathclyde A Multimodal Microscope for Improved Contrast Imaging and Optically Detected Magnetic Resonance of NV- Centres in Nanodiamond	
15:30 – 15:35	Technobite Andor	



Afternoon Tea/Coffee & Cakes; Posters; Trade

Session 4: Chair Dr Debbie Wilkinson		
16:00 – 16:15	Prof Craig Daly, University of Glasgow From Microscope to Metaverse	
16:15 – 16:30	Invited Short Talk Sali Davis, CEO Royal Microscopical Society RMS – the future	
16:30 – 16:45	Invited Short Talk Dr Simon Plummer, Micromatricies Micromatricies	

16:45 –	Prize Giving & Meeting Close
17:00	
17:00 –	Drinks Reception & Art Exhibition in the LifeSpace
18:00	Gallery
	"Boundary Laver by Kit Martin"

Everyone will leave through the LifeSpace Exhibition Space, where the drinks reception will be held from 5-6pm. Please take all of your belongings with you to the gallery, as you will not leave via the main reception.

The £50 Imaging Competition prize is kindly sponsored by Nikon. Go to <u>our website</u> to see & read about the images & videos, and remember to vote for your favourite <u>here</u>.



The £50 Poster Prize is kindly sponsored by Bruker



The £50 Oral Presentation Prize is kindly sponsored by the RMS



# JEOL-Sponsored Keynote Lecture



Dr Laura Young

**UKRI Future Leaders Fellow** 

University of Newcastle



# Imaging cells in the living human eye with adaptive optics

Laura completed her MPhys and PhD in the Centre for Advanced Instrumentation at Durham University and then took up postdoc positions in Durham and Oxford, spanning visual perception and optical instrumentation. After a Career Development Lectureship in the Department of Experimental Psychology at the University of Oxford, she joined the Biosciences Institute at Newcastle University as a Research Fellow supported by the Reece Foundation and was later awarded a UKRI Future Leaders Fellowship.

Laura's research combines optical instrumentation and psychophysical research, developing adaptive optics systems to study human vision – from imaging the light sensitive cells in the retina in vivo to studying the impact of refractive disorders on visual performance. She is passionate about interdisciplinary research, particularly the interplay between the development of new optical and imaging techniques and the generation of new understanding in the life sciences.

Her research interests lie in the functioning of the visual system, which undertakes sophisticated computations to process the vast amount of information entering our eyes. Far from being passive windows on the world, our eyes move to dynamically sample the visual scene and extract relevant information for the task at hand.

# Abstracts – Invited Presentations

# The lumicks c-trap microscope: a new facility for high resolution mechanobiology

#### Professor Carlos Penedo, University of St Andrews

The roles played by mechanical forces manifest across all biological scales, from the nanometre-size structural changes observed in proteins to the large-scale relaxation and contraction phenomena happening in muscle tissue that allows body movement. There is mounting evidence that these forces have a fundamental role in a myriad of molecular and cellular processes. In this talk, we will introduce the LUMICKS C-trap confocal microscope, a new BBSRC facility at St Andrews open to users across Scotland for high resolution molecular and cellular mechanobiology.

A crucial advantage of the C-trap is its correlative imaging capability, so that in addition to measuring endogenously generated or externally applied forces, a simultaneous fluorescence readout of the system using confocal fluorescence imaging can be performed in real time. The ultra-high stability dual-trap configuration of the C-trap allows to measure the smallest (0.1 pN), transient, changes in force with high spatial resolution over long periods of time. In addition, the C-trap includes a multi-channel laminar flow microfluidics device for rapid changing of conditions, and it can be combined with multi-color colocalization or with Förster Resonance Energy Transfer (FRET) to report nanometre-size changes in local structure. Thus, the instrument will allow researchers to directly measure the effects of mechanical forces using a multidimensional approach including force, distance, fluorescence, position and their changes over time.

## Introduction to the Dundee cryoEM facilitytailoring to the needs of the research community

# Dr Ramasubramanian Sundaramoorthy University of Dundee

Cryo-electron microscopy (Cryo-EM) has become a powerful method to determine three-dimensional (3D) structures of macromolecular complexes. Technological developments to electron microscopes, detectors and processing software have enabled researchers to achieve high-resolution structures. Hence Cryo-EM has revolutionized structural biology to provide unprecedented insights into protein structure and function. In Dundee, we have recently established a state-of-the-art 200Kv Glacios cryo electron microscope. The combination, of the improved optics of the Glacios microscope, throughput and stability of the autoloader, sensitivity, and high DQE Falcon4i direct electron detector enabled a step change at which the speed and quality samples can be screened, and high-resolution data acquired. We describe here different workflow for Glacios microscope and how users can take advantage of our CryoEM facility.

## **Microscopy in Heritage Science**

#### Dr Callum Graham Historic Environment Scotland

Heritage science concerns the scientific study of cultural and natural heritage. It is a diverse field which is based on the fundamental principles from a multitude of scientific disciplines, and successfully bridges the gap between social and natural sciences. By adopting a range of analytical techniques derived from other scientific fields, heritage scientists can help to improve the understanding, care, conservation, and management of heritage assets.

Microscopy is one of the most important and powerful techniques employed within heritage science. Ranging from low powered handheld USB microscopes used for rapid in-situ determination of stone lithologies within a building through to the use of optical, scanning electron and x-ray microscopy techniques for a more robust understanding across a range of materials and museum collection items. This talk will focus on the use of microscopy techniques within heritage science, providing interesting case studies where microscopic analysis of stone and mortar materials was key in aiding the understanding of material composition, condition, and provenance to better inform their care and protection; enlightening the human stories behind their use.

## **RMS-** The Future

Sali Davis, CEO Royal Microscopical Society

A short talk delivered by recently appointed RMS Chief Executive Sali Davis who will talk about what RMS membership covers, what the plans for the future of the RMS are in the light of the threat of Open Access publishing and the future of the Journal of Microscopy. Sali will talk about the advantages of membership of the society and the risk strategy for the next 5 years against the backdrop of an internal commitment to Sustainability, Equity, Diversity, Inclusion and Accessibility (EDI&A).

## **Micromatricies**

Dr Simon Plummer, *Micromatricies* 

Simon will give a short talk on technicalities and applications of using MicroMatrices microTMA (microtissue microarray) technology for multiplexed microscopic analysis of microtissues (organoids, spheroids). The talk will focus on how the microTMA can increase the efficiency of histological analysis of microtissues and highlight applications of artificial intelligence (AI)-based image analysis and drug-response analysis of microtissues.

## **Scottish Microscopist of the Year**

#### Dr Liam Rooney, University of Strathclyde

Liam's research focuses on innovative optical methods for imaging and microbiology, with projects developing label-free interference-based imaging techniques, creating microscope-compatible 3D culture platforms for studying soil microbes, and pioneering the use of the Mesolens for microbiology. His latest work focuses on developing 3D-printed optics, providing significant advances in open microscopy with applications in resource-limited settings.

In addition to his research successes, Liam has had a significant impact on both the research and broader communities, organising numerous research symposia and collaborating with artists and local schools for outreach. A strong supporter of early career researchers, Liam directed the launch and continuation of the RMS Early Career Award, recognising and platforming exceptional achievements by ECR microscopists worldwide. He holds positions including chair of the RMS early career committee, member of their Life Sciences Committee, RMS council, founding EDIA group member, and a Microbiology Society Champion. As a receipt of the SUSLA Forging Futures Award, Liam was able to become and Industry-Academia Ambassador, leading the organisation of IndAc'22.

In recognition of his varied contributions to the Scottish Microscopy community, Liam has been awarded £500, kindly supported by Nikon and Miltenyi Biotec.





# Abstracts – Selected Presentations

# Whole-brain light sheet imaging of behaviourally-activated neurons

Dr Cristina Martinez Gonzalez, University of Edinburgh

Cristina Martinez-Gonzalez, Kirsty J Craigie, Sally Till, Peter Kind, Nathalie Rochefort, Ian Duguid and Matt Nolan.

Centre for Discovery Brain Sciences (CDBS), Simons Initiative for the Developing Brain (SIDB), University of Edinburgh

Autism spectrum disorders (ASD) are characterised by social impairments and restricted behaviours. Activation in main brain regions involved in social and emotional processing, such as the amygdala has been assessed in ASD patients using neuroimaging and histological methods. While those studies provide information of neuronal activation, they are constrained by resolution and slicing artifacts. To overcome this, we developed RatDISCO, a novel whole-brain immunolabelling and clearing pipeline to detect proteins of interest throughout large rat brain tissue without slicing or compromising transparency, safety or tissue integrity. RatDISCO achieves immunolabelling of deep brain structures by permeabilisation that can be combined with a simple antigen retrieval step before antibody labelling, followed by stager delipidation steps to achieve optically transparent samples. To visualize neurons in the rat brain that are differentially activated during behaviour, we used RatDISCO for whole-brain immunolabeling against the immediate-early gene cFos as a proxy for neuronal activation and created an 3D atlas of the rat amygdala to register and quantify cFos-positive neurons. We then compared the cell density of behaviourally activated neurons in the amygdala of wild type animals to Fragile-X knockout (Fmr1-/y) rats as a model of ASD. Given the amygdaladependent emotional processing altered in individuals with Fragile-X syndrome, we hypothesised that cFos expression in the Fmr1-/y rat brain is altered compared to wild type (WT) in response to fear conditioning.

# The OpenFlexure Microscope: Smart microscopy for everyone with open source hardware

Freya Whiteford, University of Glasgow

F. Whiteford (1), J. Knapper (1), R. Bowman (1)(1) University of Glasgow

In an ideal world, any published experiment could be reproduced exactly from the scientific record, but in practice this is often hard or impossible. The OpenFlexure project uses open source hardware and software methodologies to address many of the challenges we face when sharing a complicated instrument. Designs, instructions, and code for the OpenFlexure Microscope are shared under open licenses, using tools and platforms developed for open software. This open documentation has been used to replicate the microscope by hundreds of people, in over 40 countries, in settings from hack spaces to super-resolution imaging labs.

The OpenFlexure Microscope is a digital optical microscope, complete with high resolution 3-axis motorised positioning and configurable imaging modes. On its own, it is a reliable instrument for brightfield or fluorescence microscopy, but it is also a platform for more complicated imaging techniques, where it provides an easy way to include the fundamentals of a microscope (imaging optics and mechanical design) in a larger instrument. We have included interfaces for automation and integration of the OpenFlexure Microscope into experiments and protocols, and are now working on higher-level software tools to ensure reliable calibration and operation. Much of this builds on our **Internet of Things** architecture, where an embedded computer in the microscope makes it easier to use and to program.

This talk will cover both the technical achievements of the OpenFlexure Microscope and the community that has grown up around the design, as well as future avenues for development.

### Searching for biomarkers of tonsillar disease

#### Megan Clapperton, University of Strathclyde

Megan Clapperton [1], Catalina D Florea [2], Tash Kunanandam [2], Margaret R Cunningham [3], Catriona Douglas [4], Gail McConnell [3].

[1] Department of Physics, SUPA, University of Strathclyde, Glasgow

[2] Department of Otolaryngology, Royal Hospital for Children, NHSGGC, Glasgow

[3] Strathclyde Institute for Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow

The palatine tonsils, located at the entrance of the oropharynx, play a crucial role in immune defence during adolescence. Recurrent Tonsillitis (RT) is characterized by repetitive bacterial infections of these tonsils. Obstructive Sleep Apnea (OSA) occurs when enlarged tonsils obstruct the airway, and recent evidence has highlighted the role of bacterial infection in OSA. We have previously shown no significant difference in biofilm presence between RT and OSA cases, emphasizing the need for alternative disease indicators.

Our study explores the cytokines IL-17C and IL-1β. IL-17C is expressed in epithelial and germinal cells in response to bacterial stimulation and it's epithelial expression has not yet been shown in tonsils. IL-1β has previously been examined as a marker for tonsil disease. We hypothesized that IL-17C could be an indicator for bacteria-related tonsillar disease and that it may be spatially coincident with bacteria. To investigate the distribution of these cytokines, we examined tonsil tissue using a combination of imaging and molecular biology techniques. Using the Mesolens alongside immunofluorescence and fluorescence in situ hybridization, we localised IL-17C to surface and crypt epithelia in over 100 tissue sections. By performing guantitative colocalization analysis, we also characterised the colocalization of both ILs with bacteria, demonstrating that while IL-1 $\beta$  is commonly colocalised with bacteria, IL-17C is not. We investigated the expression of these cytokines in fresh paediatric tonsillar tissue finding that IL-17C is significantly more expressed in both RT and OSA patients compared to IL-1 $\beta$ , establishing IL-17C as a promising marker for bacterial infection in tonsil tissues.

# Remote focusing for 2-photon microscopy to follow action potential propagation transmurally in acute rabbit heart slices

Giedrė Astrauskaitė University of Glasgow

Giedrė Astrauskaitė\*(1), Sharika Mohanan(1), Erin Boland(2), Eline Huethorst(2), Lewis Williamson(1), Ryo Kinegawa(1), Godfrey Smith(2), Caroline Müllenbroich(1)

(1) School of Physics and Astronomy, University of Glasgow

(2) Institute of Cardiovascular and Medical Sciences, University of Glasgow

Cardiac diseases are among the leading causes of death globally. Therefore, methods to investigate heart pathology in animal models are essential to develop diagnostic and therapeutic interventions. In the heart, action potentials (APs) travel through the tissue to orchestrate muscle contraction and make the organ an efficient pump. Scar tissue caused by myocardial infarction impairs electrical conduction and can cause arrhythmias. Electrophysiology in the heart can be investigated with two-photon fluorescence microscopy, which offers optical sectioning, high resolution and tissue penetration depth together with labelling biochemical function. With voltage-sensitive dyes, APs were resolved as deep as 500 µm in individual planes rabbit ventricular wall [1]. However, their transmural propagation, across the ventricle wall, has not yet been observed in real time at cellular resolution. In the rabbit heart, action potentials propagate from the endocardium to the epicardium with a conduction velocity of 30cm/s. Therefore, a rapid vertical scan is necessary. Here we present an implementation of a versatile remote focusing [2,3] module, compatible with retrofitting to commercial two-photon microscopes and capable of 300Hz axial scanning over a range of 200 µm in cardiac tissue without disturbing the sample or the sample objective. We discuss necessary system optimization to compensate for pulse broadening [4], power losses and optical aberrations. Using viable rabbit ventricular slice model [5], we present preliminary data to validate the use of remote focusing to track AP propagation transmurally. We believe that this method will allow to investigate quantitatively and at cellular resolution how scar tissue impacts cardiac conduction.

References:

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[2] Botcherby, E. J., JuÅjkaitis, R., Booth, M. J., & Wilson, T. (2008). An optical technique for remote focusing in microscopy. Optics Communications, 281(4), 880-887. https://doi:10.1016/j.optcom.2007.10.007

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[5] Watson, S., Scigliano, M., Bardi, I. et al. Preparation of viable adult ventricular myocardial slices from large and small mammals. Nat Protoc 12, 2623–2639 (2017). https://doi.org/10.1038/nprot.2017.139

## Ultrafast Microphotography at submicrosecond framing rates

Professor Paul Campbell, University of Dundee

P.A. Campbell (1,2) & M.J. Connelly (1,2)

(1) Carnegie Physics Division, University of Dundee, Dundee DD1 4HN, Scotland UK
(2) Ten Bio Technologies Inc., 150 N Research Campus Drive, Kannapolis, North Carolina 28081, USA

The concept for high-speed imaging using a rotating mirror was first mooted by CV Boys in the late 1800's and finessed in style by CD Miller in preparation for observing the first atomic weapon tests at Los Alamos during World War II. At Dundee, we exploit a camera operating on the 'Miller Principle' (a Cordin 550-62), which is capable of an upper framing rate of 4 million frames per second. Moreover, we have combined that instrument with microscope optics and optical trapping for target stabilisation [within fluid ambients] purposes. We have used this somewhat unique apparatus extensively for examination of dynamic microscopic cavitation processes driven by 1MHz [diagnostic] ultrasound exposure and the findings of that research programme, presented in the context of informing drug delivery processes, will be highlighted as a means of both introducing the technology to the masses and to stimulate the imagination of those who may desire full scene framing at reasonably high [diffraction limited] spatial resolutions and in the sub-microsecond per frame temporal regime. We will also demonstrate how this ultrafast imaging facility can be further modified and exploited to achieve in-frame nanosecond resolutions, and how multiple microscopic targets can be preferentially placed in scene using real-time-controlled holography-based optical tweezing.

### A Multimodal Microscope for Improved Contrast Imaging and Optically Detected Magnetic Resonance of NV- Centres in Nanodiamond

Rebecca Craig, University of Strathclyde

R. Craig (1), Prof G. McConnell (2), Dr B. Patton (1)

(1) Physics, University of Strathclyde

(2) Strathclyde Institute Of Pharmacy And Biomedical Sciences, University of Strathclyde

Nanodiamonds containing nitrogen vacancy (NV-) centres are fluorescent nanoparticles that do not photobleach over time, even when illuminated with high optical powers. They are small in size, and have very low cytotoxicity. These properties make nanodiamonds with NV- centres ideal for biological imaging, allowing high spatio-temporal resolution biotracking and biosensing. Nanodiamond with NV- centres offer a further advantage for biological imaging through their ability to provide a readout of relative changes in magnetic field and temperature using optically detected magnetic resonance (ODMR). They have been used as a marker in various super resolution microscopy techniques but these methods are highly complex and are not routinely available in biology labs.

To lower the barrier to entry to this useful technology, we have developed a multimodal microscope specifically for imaging of nanodiamonds in biological specimens using low-cost components where possible. Our new imaging platform incorporates both widefield epifluorescence illumination for more accessible characterization of nanodiamonds and measurement of ODMR prior to their application in biological systems, and total internal reflection fluorescence for improved contrast ODMR and bioimaging using fluorescent nanodiamonds as reporters.

I will give an overview of the system design, showing imaging data obtained using the platform, and I will present progress on measurements in biological systems.

### From Microscope to Metaverse

#### Professor Craig Daly, University of Glasgow

C.J. Daly, M Koponen, D.I. Hughes University of Glasgow

Metaverse (by Meta), ViveVerse (by VIVE), Omniverse (by Nvidia): these are the 3 main visions of where we might be headed with extended realities (XR). There is no doubt that rapidly developing technologies and software make it easier to engage with virtual, mixed and augmented realities (collectively, XR). However, creating XR scenes from microscope-based data still presents a variety of challenges. This presentation will showcase two XR applications we have constructed at XR Life Science (XRLS, UofG): "A spinal cord journey" generated from serial electron micrographs, and "Structure of a cardiac myocyte" derived from confocal data. Both scenes have been extensively demonstrated at a variety of public engagement events and have consistently attracted excellent feedback. The key challenges in constructing such scenes relate to segmentation of source image volumes and iso-surface (mesh) optimisation. We have used a variety of 3D techniques and trained neural networks to improve the workflow and speed the content creation process. Both scenes contain mainly microscope derived data alongside manually created content and protein structures derived from the Protein Data Bank. There is a lack of appropriate XR content for teaching life science within higher education. However, there is an existing wealth of raw material in the form of 3D image data from which XR content could be created. Examples of such data can be found at the XRLS Sketchfab page (https://sketchfab.com/GLS/models). The presentation will cover the development of two XR-scenes and hopefully encourage microscopists to explore the potential of XR in their own studies.

Join us for the drinks reception 5 – 6pm in the LifeSpace Gallery to view Boundary Layer



Boundary Layer takes a close look at mosses and the microscopic worlds that surround them. Some of these structures and organisms are just out of our visual reach and have been made visible using both the confocal and a simple 1970s student microscope. Others can be seen just by looking very closely. The role of these modest plants, particularly sphagnum, in storage of both water and carbon is huge. Mosses help fight air pollution, colonise bare ground paving the way for other plants to grow and can reduce the risk and severity of flooding.

The work has emerged from a year spent investigating small things and different materials and processes with the MFA ART, Science & Visual Thinking at DJCAD. This mixed media exhibition includes film, photography, sculpture and installation and will be on from **9 Oct - 10 Nov**.

All are welcome to an open evening with drinks on Thursday 2 Nov 6-8pm.

# **Trade Exhibitors**

We would like to thank the following companies for supporting this meeting by exhibiting in the trade show







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The University of Edinburgh in conjunction with Bruker Nano Surfaces Metrology would like to invite you to our Nanoscale Characterisation Workshop - covering the <u>NanoWizard 4 XP</u> & <u>MultiMode 8-HR</u>, being held at the Centre for Science at Extreme conditions (CSEC), Erskine Williamson Building, on the 28th & 29th of November.

The goal of the workshop is to share the latest developments and capabilities of the Scanning Probe Microscopy for studying micro and nano-chemical, nano-electrical and nano-mechanical properties of surfaces. The workshop will be valuable for Researchers (from students, post docs to PI's), experimental officers, lab managers and other scientists involved in material characterization at the nanoscale.

The day will combine talks with practical sessions on BioAFM & Bio Materials along with nanoelectrical measurements in air and fluid. Discussions will cover a wide range of AFM applications and nanoscale characterisation.

This will also be a great opportunity to bring samples along for measurements and analysis. And you are invited to submit an image for our Image Competition! You have the chance to win a prize and that your image will be displayed in our Applications Laboratory.

Click the buttons below to register and to download the programme:

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Please contact us if have any questions regarding the event: Alison Kelly, <u>Alison.Kelly@bruker.com</u> Stephen Lewandowski, <u>stephen.lewandowski@bruker.com</u>

# S MC

#### Strathclyde Optical Microscopy Course 4-9 August 2024

#### University of Strathclyde Glasgow United Kingdom

Organisers: Brad Amos, Gail McConnell, Liam Rooney

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Lecturers: Brad Amos, Gail McConnell, Bob Edkins, Susan Cox, Siân Culley, Lucy Collinson, Ralf Bauer, Liam Rooney

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