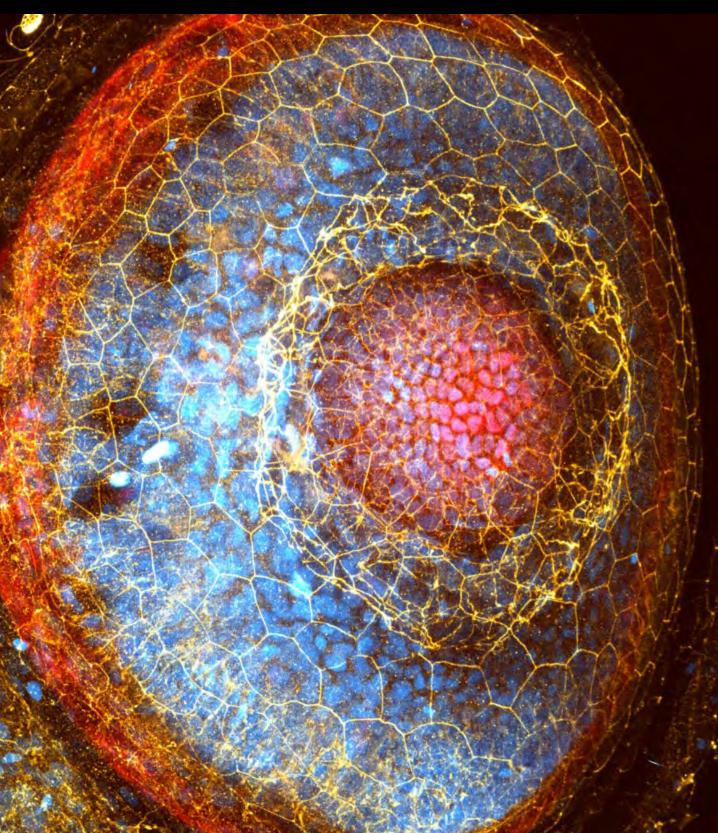
ANZSCDB

Australia and New Zealand Society for Cell and Developmental Biology Inc.





SUMMER NEWSLETTER 2021-2022

ANZSCDB

Australia and New Zealand Society for Cell and Developmental Biology Inc.



SUMMER NEWSLETTER - January 2022

President's welcome statement

Dear ANZSCDB members,

My ANZSCDB journey started in 2006, soon after I returned to Australia from a postdoc in the US. I had started my new lab in Melbourne at the Peter Mac, was eager to recruit students and staff and forwarded a job ad to ANZSCDB for circulation among members. It's fair to say at that point, that I was looking for how ANZSCDB could help me. Alpha Yap was President at the time and happily obliged, but also suggested I join the society.

In this issue

- President's welcome
- 2021 Award winners
- New Exec and State Reps
- News and Activities
- Publication highlights
- Meeting reports
 - SA meeting
 - VIC meeting
 - Fly meeting

Since that time, ANZSCDB and its members have been a very influential and supportive part of my scientific life. I have made many friends, enjoyed interactions at conferences like Combio and the Hunter Meeting and been fortunate to receive crucial mentorship and support. My science spans fundamental and applied biology but I feel most at home in a group of cell and developmental biologists - ANZSCDB has always felt like "my tribe".

The society has already offered me many special opportunities; three that stand out are: advocating for research with politicians at Science Meets Parliament (with Edna Hardeman, in 2007), kicking off the Victorian chapter of the state-based ANZSCDB meetings (with Ian Smyth, in 2010), and learning how to use Excel and balance the books as Treasurer (under the presidency of Edna and then Peter Currie). Each of these opportunities gave me the chance to learn new skills and meet and work with talented colleagues.

It also allowed me to become familiar with some of ANZSCDB's great qualities: a focus on scientific excellence, providing opportunities to meet colleagues and share and discuss scientific ideas, supporting members at all career stages, and advocating for scientific research.

I am now very honoured to serve ANZSCDB as its President. Going before me are some truly exceptional scientists and people who I have admired for years, so there are big shoes to fill. Knowing that, my first job was to recruit a committed and enthusiastic executive and I am thrilled to be joined by Jennifer Zenker, who will serve as Treasurer, and Alex Combes, who will serve as

Secretary, both from Monash University. To steal both an ethos and a great word from Pete Currie, I plan to govern ANZSCDB as a triumvirate. Jenny and Alex have already come up with a great list of new initiatives and we hope to deliver on these as we emerge from the pandemic-induced lockdowns that have kept us apart.

I want to thank the former ANZSCDB executive Jenny Stow, Sam Stehbens and Nathan Pavlos and acknowledge their dedication and hard work. The past 2 years have been very difficult for everyone but despite the many trials they have faced, Jenny, Sam and Nathan have successfully navigated the society through several challenges. We look forward to working with them through the interregnum period (thanks Michael Samuel for another great word!) and carrying on their work.

Finally, if you have any ideas for how ANZSCDB can best serve its members, please contact me. I hope to see you all in person soon.

Sincerely,

Kieran Harvey

ANZSCDB President

Cover art

Cellular complexity in the zebrafish eye, Dr Ivar Noordstra (IMB, University of QLD), see Awards section for more information.

Keeping up to date

Remember to follow <u>@ANZSCDB</u> on Twitter for news and tag us in your work-related posts for re-tweets.

Would you like to contribute to the ANZSCDB newsletter?

Please send items to <u>Alex Combes</u>, the society Secretary, or get in touch with your <u>state representative</u>.

The newsletter will be published approximately every three months and distributed to all ANZSCDB Members via e-mail. Previous newsletters are hosted on our website.

Please ensure that your submissions are succinct and have been fact-checked.

ComBio2022

27th September to 30th September 2022

Registrations open April 2022

Please register as an ANZSCDB member to help support the society



Melbourne Convention and Exhibition Centre

South Wharf, MELBOURNE 27 September - 30 September 2022

https://www.combio.org.au/combio2022/

Hunter 2022



THE HUNTER MEETING 2022 @ THE NEX, NEWCASTLE

https://www.huntermeeting.org.au/

ANZSCDB Award Winners 2021

The society is proud to celebrate the impactful science, inspiring leadership and innovative ideas of its award winners for 2021. We will be looking forward to new applicants for the 2022 awards. Please see the ANZSCDB <u>website</u> for more information on the 2021 winners and the award categories.

The 2021 President's medal and Emerging Leader talks will be presented at an ANZSCDB Symposia to be announced shortly.

ANZSCDB President's Medal Award

Prof John Bertram, Department of Anatomy and Developmental Biology, Biomedicine Discovery Institute, Monash University, VIC

John is internationally recognised for his many contributions to our understanding of the developmental programming of kidney development, and the consequences for adult health. He and his colleagues reported the most significant findings to date on human nephron number and links with birth weight, race, hypertension, and chronic kidney disease. The



findings from studies on 6 human populations, including indigenous and white Australians, provide the strongest evidence to date that low birth weight driving low nephron endowment increases the risk of chronic disease. John's research leadership and pioneering spirit are an inspiration to our Society and we are grateful for his ground-breaking contributions to ANZSCDB and the international research community.

ANZSCDB Emerging Leader Award

A/Prof James Murphy, Pseudokinase lab, Walter and Eliza Hall Institute of Medical Research, VIC

James has developed an extensive body of work performing detailed studies of the MLKL pseudokinase to understand the mechanisms underlying its activation and how it kills cells via the lytic cell death mode, necroptosis. Necroptosis has ancestral functions in host defence but has emerged as dysregulated in a wide range of inflammatory human diseases. His research thus seeks to unravel the signalling control of this process and its role in



disease. James has established himself as a leading researcher in the competitive field of cell signalling, securing continuous funding from the NHMRC in project and investigator grants and high impact publications in leading international journals. We are extremely pleased to award this prize to James to recognise his contribution to our Society and the wider scientific community.

ANZSCDB Early-Career Researcher Award

Dr **Max Nobis,** Invasion and metastasis lab, Garvin Institute of Medical Research. "We use intravital FLIM-FRET imaging to explore signalling cascades in development and cancer, such as small GTPase signalling RhoA, Rac1 and kinases such as Src and Akt, and employ optical window imaging in spatiotemporal monitoring of treatment response to targeted inhibition of cells in their native microenvironment"



ANZSCDB Image Awards

From the cover: I Spy With My Little Eye, Dr Ivar Noordstra, Institute for Molecular Bioscience, The University of Queensland. "We use zebrafish to study the biological processes underlying the perception of light. This image highlights the fascinating complexity of the zebrafish eye. It consists of multiple cell types including epithelial cells (red), endothelial cells and neurons. All these cells are tightly linked together through specialized protein complexes like tight junctions (yellow). The combination of junctional and nuclear staining (syan) heautifully illustrates the incredible diversity of



staining (cyan) beautifully illustrates the incredible diversity of cell shapes."

Below: Three-dimensional alveolar architecture in an involuting murine mammary gland, Dr Krystyna Gieniec, Single Molecule Science, University of New South Wales. "I am investigating the role of calcium signalling in the function of star-shaped, mammary basal cells as they transition through gestating, lactating and involuting states. Additionally, I am examining the calcium signalling cross-talk between mammary epithelial cells and their immediate cellular environment during gland embryogenesis and tumorigenesis."

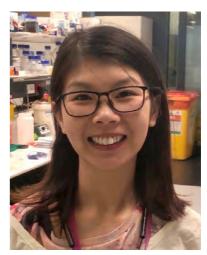


ANZSCDB Publication Award

Dr Qian Dong, Peter MacCallum Cancer Centre, VIC

Glial Hedgehog signalling and lipid metabolism regulate neural stem cell proliferation in Drosophila doi.org/10.15252/embr.202052130

"The glial niche regulates the proliferation of neural stem cells in the Drosophila larval central nervous system (CNS). My research showed that glial Hh signaling autonomously facilitates cortex glial niche formation, and non-autonomously regulates neural



stem cell proliferation dependent on the lipid regulators Fasn1 and Lsd2."

Get to know your new Exec and State Representatives



ANZSCDB @ANZSCDB · Nov 19

Please join us in welcoming ANZSCDB's new executive team! President:
Kieran Harvey @HarveyHippoLab; Treasurer: Jennifer Zenker @LabZenker;
Secretary: Alex Combes @AlexKoomz. We look forward to your leadership as you steer ANZSCDB to new heights!



Our website has been updated to include profiles the Executive new Committee, and for our new and continuing State <u>Representatives</u> extend a special welcome to State our new Representatives Nathalie Dehorter (ACT), Emily Don & Qian (Peter) Su (NSW), Ivar Noordstra & Merja Joensuu (QLD), Anna

Oszmiana & Yasmyn Winstanley (SA), Brooke Huuskes & Jan Manent (VIC).



News and Activities

The Journal of Cell Science is seeking submissions from researchers at any career stage whose experiences as a cell biologist have been shaped by equity, diversity and/or inclusion. See the journal website for more information about this <u>ongoing essay series</u>.

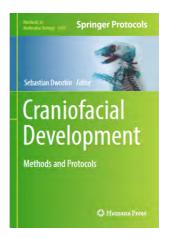
Congratulations to Kazuhide Shaun Okuda for taking out the Life Science category of the Light Microscopy Australia image competition with his stunning wholemount images of <u>ribosomal biogenesis and vasculature in developing zebrafish embryos</u>.

Publication highlights from members

The **Zenker** lab at ARMI, Monash University has recently published a Review in *Development* titled Microtubule-dependent subcellular organisation of pluripotent cells. This Review details discoveries in microtubule organisation of early embryos and elucidates how the cytoskeleton-organelle interactome is an integral contributor to pluripotent cell identity in vivo and in vitro. https://doi.org/10.1242/dev.199909

Sebastian Dworkin, La Trobe University, has edited a Methods and Protocols book in Craniofacial Development (Cover pictured right)

A joint research project co-led by Professor **David James** and Dr **Sean Humphrey** from the University of Sydney and Professor Jørgen Wojtaszewski from the University of Copenhagen, could revolutionise our understanding of type 2 diabetes in diverse populations and provide new drug targets, initiating the development of Precision Medicine strategies to tailor appropriate treatments. Exercise is



known to have profoundly beneficial effects for people with diabetes as it improves insulin sensitivity. However, the mechanisms for this effect have eluded researchers for decades. By teaming up with researchers in Copenhagen, world leaders in exercise research in humans, James and Humphrey have applied their cutting_edge mass spectrometry methods to study protein phosphorylation in people following exercise. One of the striking findings to emerge from this work is that each individual was found to possess a unique pattern of protein phosphorylation spanning many thousands of data points. By utilising these unique patterns they were able to for the first time begin to discover novel aspects of the molecular circuitry used by exercise to encode its beneficial effects. Read the article in *Nature Biotechnology* here https://doi.org/10.1038/s41587-021-01099-9

The **Hogan** lab at Peter MacCallum Cancer Centre has published a breakthrough study on the role of the Ddx21 RNA helicase lymphangiogenesis. Read about it in Nature Cell Biology https://doi.org/10.1038/s41556-021-00784-w

The **Rosello-Diez** lab at ARMI has developed a new pipeline to segment and measure bone length in 3D models obtained by computed tomography. Check it out in *Frontiers in Cell and Developmental Biology* https://doi.org/10.3389/fcell.2021.736574

Nathan Palpant and colleagues have published a review on single cell methods for studying stem cell differentiation in *Trends in Molecular medicine* https://doi.org/10.1016/j.molmed.2021.09.006

2020 ANZSCDB President's Medalist **Peter Gunning**, ANZSCDB Committee Member **Edna Hardeman** and colleagues at UNSW have published a breakthrough study on the critical role of actin/tropomyosin filaments in cancer cell adhesion and mechanosensitivity of proliferation. Check out the <u>pressrelease</u> or read the paper in *Nature Materials* 10.1038/s41563-021-01087-z

Get in touch to highlight your papers in the next newsletter or via the @ANZSCDB Twitter account.



9th ANZSCDB Adelaide Meeting Report

The 9th ANZSCDB Adelaide meeting was held on 19 October 2021, within the Centre for Cancer Biology, UniSA. There were a record number of registrations as well as abstracts submitted for this meeting, with 70-75 in person attendees and 20+ Zoom participants! Over 20 new members joined the ANZSCDB in preparation for this meeting.

Invited Prof speakers Ruth Arkell (Australian National University), A/Prof Archa Fox (University of Western Australia) and Dr Yoon Lim (Centre for Cancer Biology) gave amazing talks and stimulated great discussions durina question time and breaks. In addition, our postdoc and student oral presentation



sessions were of outstanding quality, making it difficult for the judges! In a first for this meeting, we featured an online session of interstate presentations selected from abstracts, which received very good feedback. In addition, we had a very busy and noisy lunchtime, with 24 poster presenters showcasing their research.



We are very appreciative of all the support we received from sponsors (indicated below) and the ANZSCDB, which allowed us to provide free registration, catering and multiple prizes to presenters. Trade displays and short virtual presentations from sponsors were well received. Thanks to their generous support, we were able to award 13 prizes! We would like to congratulate the following prize winners: Best Postdoctoral Oral Presentation winners Sophie Wiszniak

and Yasmyn Winstanley, Best Student Oral presentation winner Ellen Potoczky and Runner-up Emmylou Nicolas, Best Postdoctoral Poster Presentation winner Jan Kazenwadel and Runner-up Sandii Constable, Best Student Poster Presentation winners Emma Cheney and Ashleigh Geiger and Runner-ups Erica Kolze and Ammara Usman Farooq and Best Research Image winner Saba Montazaribarforoushi and Runner-ups Anna Oszmiana and David Herrmann. The event was concluded by drinks and networking which was the best way to end the day. We look forward to what the ANZSCDB Adelaide meeting brings in 2022!

Report by ANZSCDB SA State Representatives and meeting convenors, Jantina Manning and Winnie Kan





13th ANZSCDB VIC State Conference report

Over 100 Cell and Developmental Biologists from all over Australia were "travelling" to the 13th ANZSCDB VIC State Conference – taking the same route, from bed to laptop. What seemed to be first a big disappointment being forced changing to a virtual format, turned out to be a very stimulating and successful day for all attendees.

The inspirational talks from ANZSCDB President's Medal Winner 2020 Prof. Peter Gunning and ANZSCDB Emerging Leader Award Winner 2020 Prof. Michael Samuel about their career and science was embedded in cutting-edge scientific talks by PhD students and PostDocs from University of Melbourne, Monash University, WEHI, Peter MacCallum Cancer Centre, La Trobe Institute and Murdoch Children's Research Institute. The poster presentations were converted to flash talks organised by topic in 5 breakout rooms: 1) Tissue patterning and Development, 2) Cell and Molecular Biology, 3) Cardiac Biology, 4) Neuromuscular Disease and Metabolism and 5) Health and Disease.

Huge congratulations to all ANZSCDB award winners of the day who delivered outstanding presentations and are representing the upcoming generation of world-leading scientists in Australia (pictured below). A big thank you also to our sponsors: University of Melbourne, Australian Regenerative Medicine Institute (ARMI), The Walter and Eliza Hall Institute (WEHI), BMG Labtech, Scitech, ATA Scientific, VectorBuilder, Sartorius, Cartherics and Monash Genome Modification Platform, who made it possible to organise a free conference for all ANZSCDB members and non-members during such challenging times.

The conference was organised by a very enthusiastic group of people who volunteered to dedicate some of their valuable time to ANZSCDB: Andre Samson, Dagmar Wilhelm, Jan Manent, Alex Combes, Avnika Ruparelia and Jennifer Zenker.

Report by Jennifer Zenker



Another great meeting 'flies' with the support of ANZSCDB

By Teresa Bonello

Two years into a pandemic, with many of us suffering from extreme Zoom fatigue, the prospect of an in-person scientific meeting is an exciting one. Particularly when the meeting includes the picturesque backdrop of a quiet country town, away from the sensory overload of the city. For the Australian fly community, Warburton, in rural Victoria has been that place of scientific sanctuary. Every year, fly biologists from across Australia descend on the Yara Valley to catch-up with colleagues, old and new, talking science within the walls of the historic Alpine Retreat. While it was clear 2021 would not bring that welcome relief, it was also a stark reminder that our rural communities, many of which depend on out-of-town visitors and hosting meetings such as ours, were some of the hardest hit businesses in Australia during this time.

Faced with another online meeting, the fly community was not deterred. This year brought with it record attendance for the Australian Fly Meeting, which was held last month. With the support of ANZSCDB, we were able to come together online, but with a twist. Satellite meet-ups took place in Queensland, South Australia and Tasmania, with each location seamlessly integrating into our online platform. It seems that after two years we have all become audio-technical specialists in our own right.

Plenary speaker of the day was Professor Tatsushi Igaki from Kyoto University, who spoke about his fascinating work with 'winner' and 'loser' cells. He told us that certain oncogenic mutations are known to alter the fitness of cells, turning them into supercompetitors where they can eliminate their neighbours. This type of cell competition has been linked to tumour progression. It was wonderful to hear Igaki, a world leader in the cell competition field, recognise the Australian scientists who had also shaped this field and continue to do so.

One of the unique aspects of the Australian Fly Meeting is that it serves to showcase and share research on behalf of the collective research group, not just the individual. This means everyone participates – with an emphasis on getting our most junior scientists involved. Thanks to ANZSCDB and the John Curtin School of Medical Research we were able to recognise the remarkable progress that our students have continued to make in their research, despite ongoing roadblocks. Sarah Mele, PhD student at Monash University, in the lab of Dr Travis Johnston and Dr Matt Piper, was one of the awardees this year. Sarah discussed her fly model for a human genetic disorder involving inborn errors of amino-acid metabolism. Her work is a beautiful example of how *Drosophila* can be used to model complex physiological conditions. Masters student Amber Kewin, supervised by Dr Sean Millard at the University of Queensland, impressed us with her findings on Amyotrophic Lateral Sclerosis in the fly nervous system. Her presentation was a molecular tour de force, resolved from the stunning visualisation of neurons that imaging in *Drosophila* so uniquely affords.

The Australian fly community is wonderfully diverse, with talks of the day ranging from odorant receptors in the Australian sheep blowfly population down to the elegantly organised subcellular forces acting across epithelial tissue. Despite our diverse interests, engagement across the community remains at an all-time high. What unites us? Perhaps, it is simply our common appreciation for the powerhouse of biological information that resides within the humble fruit fly. Yet, it is more than that. There is a distinct sense of generosity within the fly community that can be noticed both here

and abroad. A culture of sharing resources, knowledge and support, that keeps us connected even (especially) in the midst of a pandemic.



Talk prize awardees Sarah Mele (top, left) and Amber Kewin (bottom) with meeting organisers (Matt Piper, Rippei Hayashi and Teresa Bonello)

ANZSCDB Corporate Member News:

We would like to thank the following corporate sponsors. Please visit their websites below and peruse their advertisements at the end of this newsletter.

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Thanks to the following sponsors for their support of our state meetings.

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SA Meeting Sponsors:

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- DKSH
- Lonza

VIC Meeting Sponsors:

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- Monash Genome Modification Platform



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LIVECYTE: A VITAL TOOL FOR PROSTATE CANCER PRIMARY CELL ANALYSIS

The need for more complex cellular models for prostate cancer research

Current prostate cancer cell lines are incapable of accurately modelling the behaviour of prostate tumours due to loss of the complex cell signalling pathways present in heterogeneous cultures. Phenotypes between patient cancer cells can also vary wildly and in a multitude of ways. This variation is not reflected in established cell lines – emphasising the need for more complex cellular models for developing and screening cancer treatments.

To address the cell line problem, Professor Norman Maitland's research group at the University of York looked at using primary prostate cells to study new cancer treatments. This allowed them to study cellular interactions within a tumour and represent different, more clinically relevant, cancer types.

Limited options for analysing heterogeneous cell cultures

In analysing primary prostate cancer cells, researchers found that established options for characterising cells in a heterogeneous culture are suboptimal for gaining accurate data. Initially they looked at exposing a population of cells to the hypothesised treatment and then separating subtypes into individual cultures, which was laborious and led to changes in cellular activity. The alternative option was via fluorescent labelling, which leads to phototoxicity caused by release of reactive oxygen species.

They sought to use Livecyte, a label free high content kinetic cytometer, to identify their different subpopulations within the heterogeneous culture using its quantitative phase imaging modality.

Livecyte: Automated cell tracking and analysis

Livecyte produces high contrast label-free images of cell culture using ptychography. Automated analysis algorithms produce accurate segmentation of each cell, allowing investigators to automatically track individual cells and extract valuable information about their behaviour such as

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motility, proliferation, and dry mass.

Using Livecyte, researchers were able to illustrate the behavioural disparity between commonly used cell lines and primary cells. This that heterogeneity cannot adequately be represented by single cell lines during treatment development. They demonstrated that primary cells were larger, and faster, however increased in dry mass and cell count at a lower rate than the lines (Figure 1). These differences in growth and proliferation would impact the duration of time treatments would take to be effective.

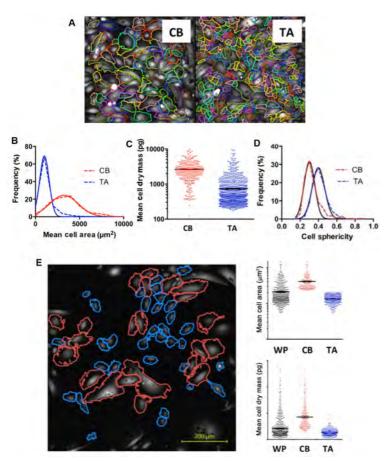
Figure 1: Livecyte imaging shows that primary prostate cultures divide less frequently than cell lines but undertake significantly more movement in 2D culture. A panel of prostate cell lines was grown alongside a primary prostate epithelial culture and time-lapse imaging was carried out. (A) Brightfield images of each cell type; (B) Livecyte images of each cell type with cell segmentation

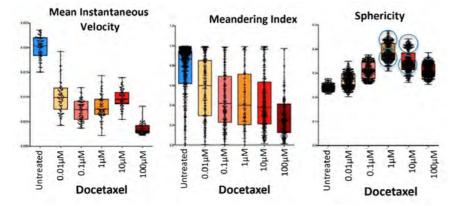
outlines (coloured lines) and cell tracking ID (coloured numbers) shown; (C) 2D representation of tracking of each cell type (X-axis, x position; Y-axis, y position); (D) 3D representation of tracking of each cell type (as for 2D but including a Z-axis, time); (E) mean

cell area is plotted for each cell type. Each dot represents a single cell track; (F) mean speed is plotted for each cell type. Each dot represents a single cell track; (G) the total dry mass of each frame of the time-lapse video is plotted, which is indicative of cell growth and proliferation.

Livecyte: An even greater depth of analysis

The team were further able to distinguish subpopulations of Transit Amplifying (TA) cancer progenitor cells and Committed Basal (CB) differentiated cells taken from primary culture. TA cells are an active component of tumours, where abnormal differentiation causes cell accumulation resulting in cancer growth. The characteristics of isolated TA and CB cell populations were analysed on Livecyte, finding that CB cells were less spherical, and larger in size and dry mass than the TA cells. Unique cellular fingerprints were developed for both cell types from these findings, leading to categorisation of CB and TA cells within a heterogeneous culture (Figure 2). The team could then observe heterogeneous primary cell dynamic responses to a Docetaxel treatment, label-





free in real time. This proves the possibility of rapidly screening and personalising prostate cancer treatment from patients' biopsied tumours.

Figure 2: Signatures of two populations of cells within primary prostate cultures can be characterised from Livecyte data and used to identify different cell populations within heterogeneous cultures (A) Livecyte images of TA and CB cells showing cell segmentation outlines (coloured lines). Data from Livecyte analysis of each cell type was measured including (B) mean cell area, (C) mean cell dry mass and (D) cell sphericity; (E) analysis of a mixed culture of cells with gates applied to separate out the two cell populations based on cell area. Data from the whole population (WP) and each cell type was measured and plotted as mean cell area and mean cell dry mass.

Additional work investigated the changes in primary cell morphology upon dosing with Docetaxel. Increased dosage led to a decrease in velocity and meandering index, as well as an increase in sphericity as cellular mitosis was disrupted. The team discovered bi-modal and tri-modal responses in cell sphericity indicating

drug resistance in small subsets of cells; some cells flattened and died, whereas others maintained their sphericity and survived (Figure 3).

Figure 3: Dynamic responses of primary prostate cells to Docetaxel can be extracted from the Livecyte data. Each cell is measured, and patterns of response recorded. Bimodal sphericity responses are observed with some parameters that can be related to the cell behaviour

(turquoise circles).

Upon observing the time-lapse images, outlier cells could be identified. These cells moved more erratically, became spherical upon attempting mitosis, and kept moving after failing division.

Further work is needed to confirm the resistance of these outlier cells, however, if this is the case, cells which could ultimately lead to post-treatment cancer recurrence could be identified. In treating every cell as a data-point, Livecyte was able to reveal details otherwise masked with more traditional assays which look at the average of a population.

Conclusion

In analysing primary prostate cancer cells to study new cancer treatments researchers determined that established options for characterising heterogeneous cultures, such as fluorescent labelling or separating subtypes, led to changes in cellular behaviour. Using Livecyte, however, they were able to perform long-term timelapse imaging whilst automatically segmenting and tracking cells in their heterogeneous prostate cell cultures. They could distinguish subpopulations of TA and CB differentiated cells and quantify their behaviour, independently and whilst cocultured, in response to Docetaxel treatment. Livecyte identified bi-modal and tri-modal responses in primary cell morphology to Docetaxel pointing to drug resistance in a small subset of cells. Livecyte opens the realms of possibilities within cancer research, enabling investigators to identify therapy resistance in individual cells. Furthermore, it takes us considerably closer to the goal of rapid screening of treatments and personalisation of therapies to cancer patients, a prospect which would improve many millions of cancer prognoses.

Reference: https://www.phasefocus.com/resources/customer-testimonials/York-vital-tool-prostate-cancer-primary-cell-analysis

For more information contact us

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