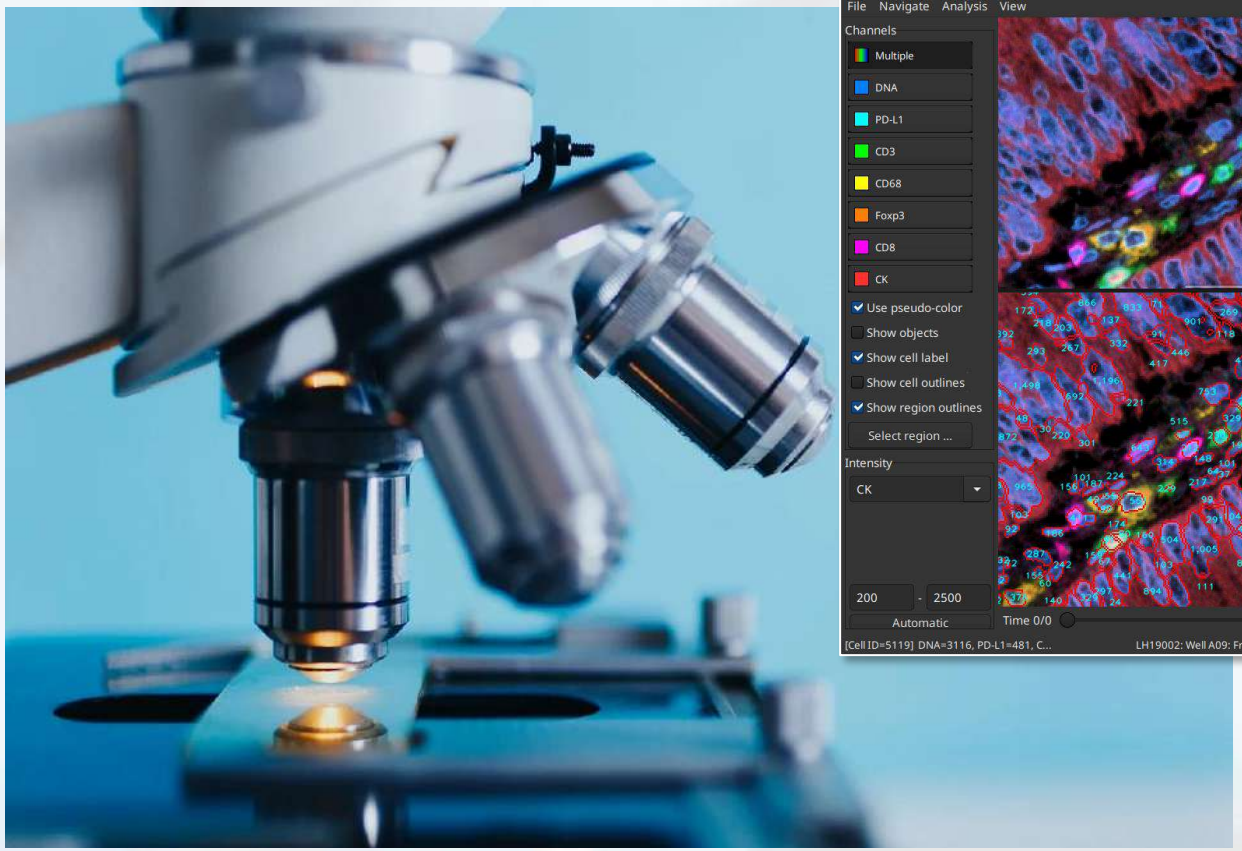
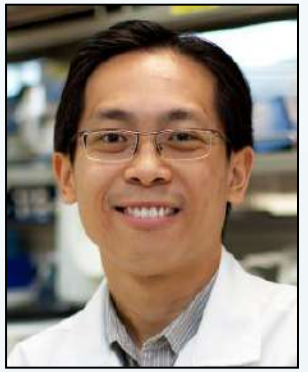
Complex Cellular Phenotype Analysis

Bioinformatics Institute
BII

CREATING GROWTH, ENHANCING LIVES





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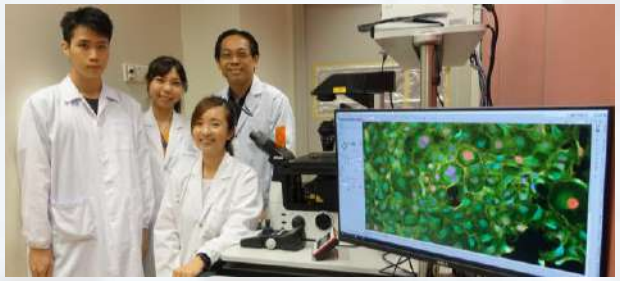
HPAShare
View and share anywhere, anytime

ImmunoAtlas

Complex Cellular Phenotype Analysis Group, BII



Phenotypic profiling and computational modeling

- Chemical/drug safety or efficacy assessments
- High-throughput Image-based Phenotypic Profiling
- Machine learning, data analysis, and assay automation

ToxMAD

Toxicity Mode-of-Action Discovery Platform

Assess chemical safety based on mechanistic reasoning

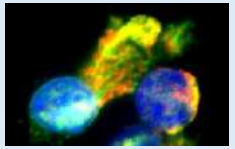
Bioimage Databases

HPAShare
HPAScore
ImmunoAtlas

View and share anywhere, anytime

Big bioimage data management, visualization, standardization, and analytics

Digital Medicine for Cancer



Data-driven cancer treatment and care

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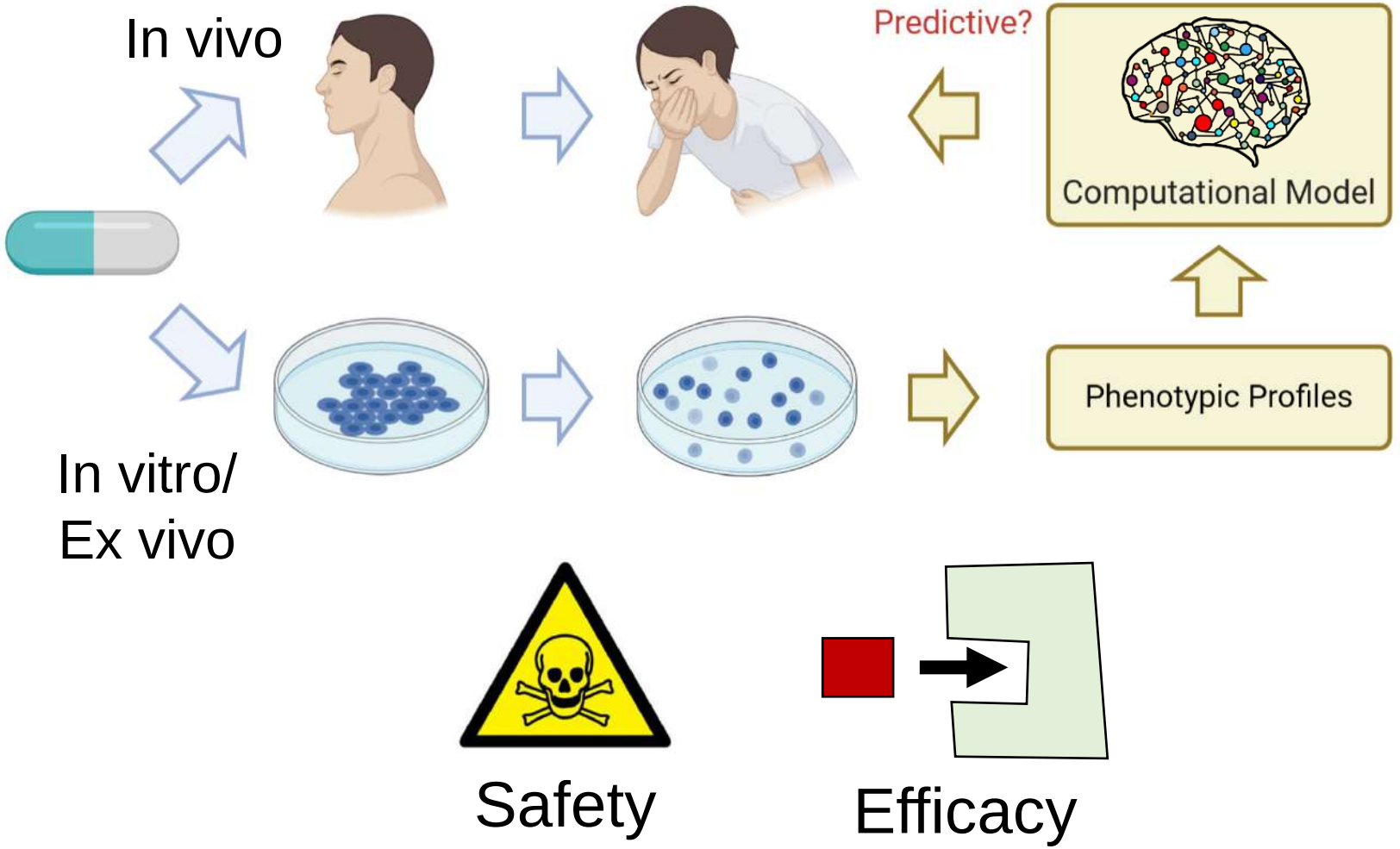
Building Predictive In vitro or Ex vivo Models for Chemical/Drug Safety or Efficacy Assessments



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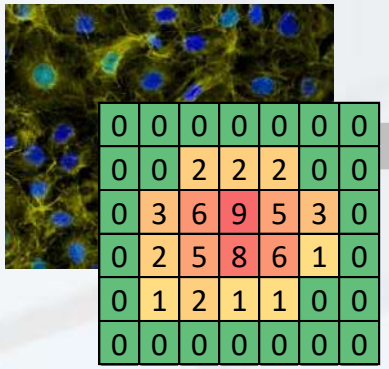
High-throughput Image-based Phenotypic Profiling (HIPP)



Measure **large** numbers of phenotypic features based on **general markers**

Select the most "predictive" feature subset

Build and evaluate computational models

Conceptually similar to "RNA expression profiling"

	Phenotypic Profiling	High-Content Analysis (HCA)
Number of measured phenotypic features	Very Large (~100s-1000s)	Small (<10)
How are the features designed and selected?	Automatically based on machine-learning algorithms	Manually based on known or expected mechanisms
What stains/markers are used?	General cell structures or biological processes	Specific structures or biological processes
Modes of action need to be defined a priori?	No	Yes
Can discover novel MoAs?	Yes	No

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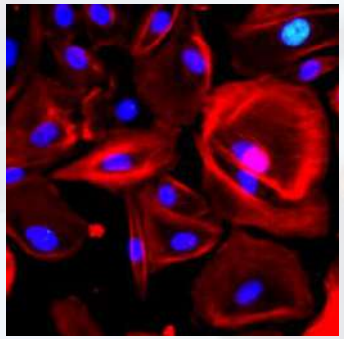
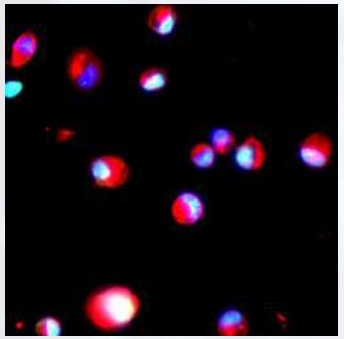
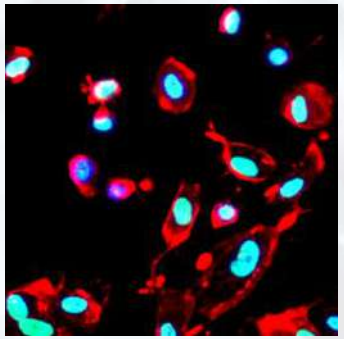
Proximal tubule cells exhibit distinct phenotypes when exposed to different chemicals

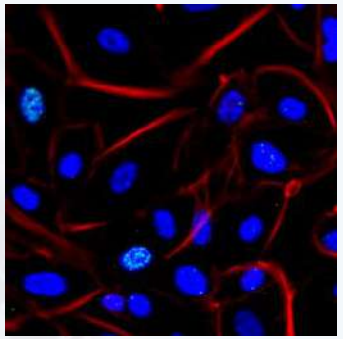
Non-PTC-Toxic reference chemicals

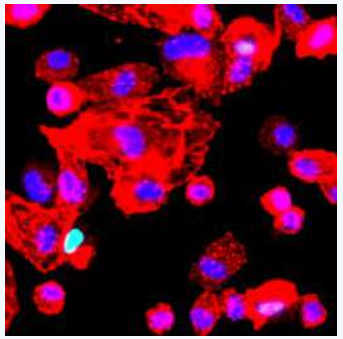
PTC-Toxic reference chemicals

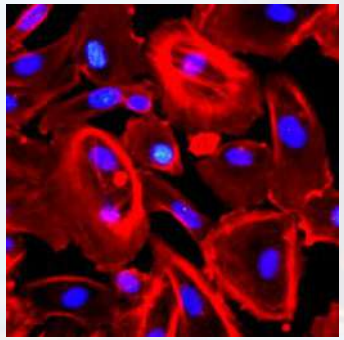

Dexamethasone

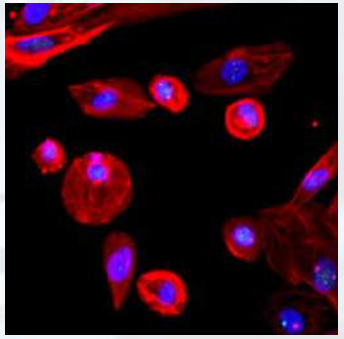

Arsenic Oxide

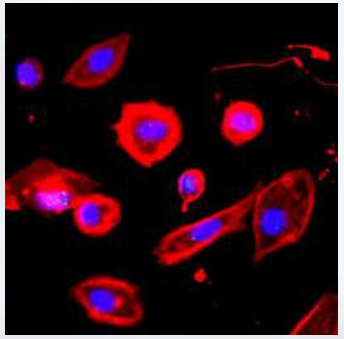

Cephalothin

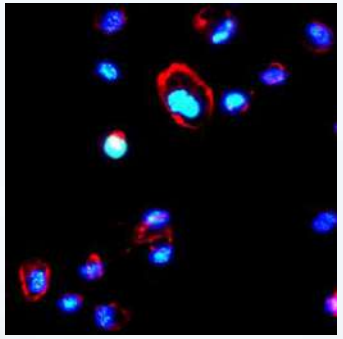

Paraquat

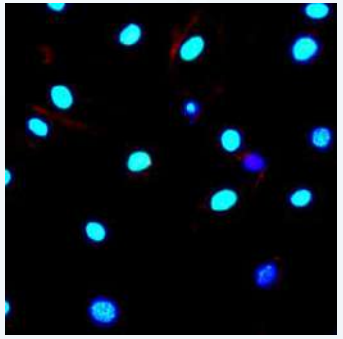

Potassium Dichromate


Water


Tetracycline


Bismuth Oxide


Cadmium Chloride


Gold Chloride

Human PTCs were treated with 44 reference chemicals and stained with DNA, Actin, RelA/gH2AX

In collaboration with Dani Zink, SIFBI

[Su et al., Arch Tox, 2016]

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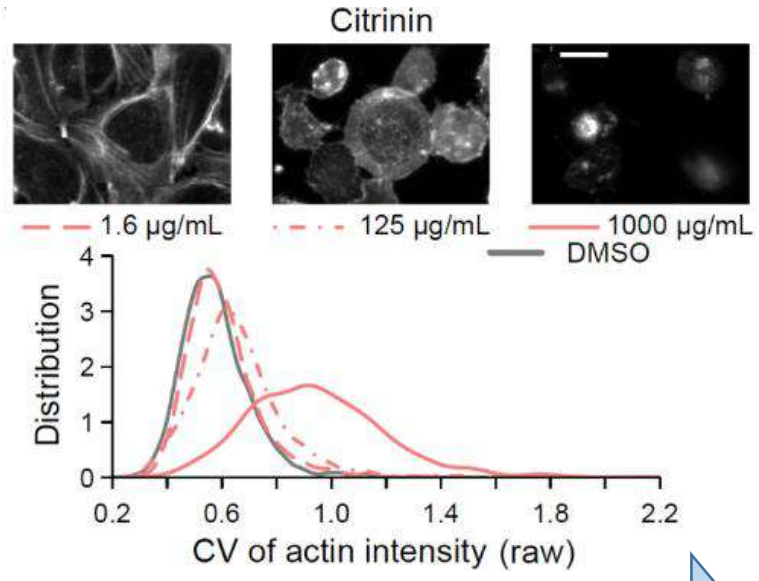
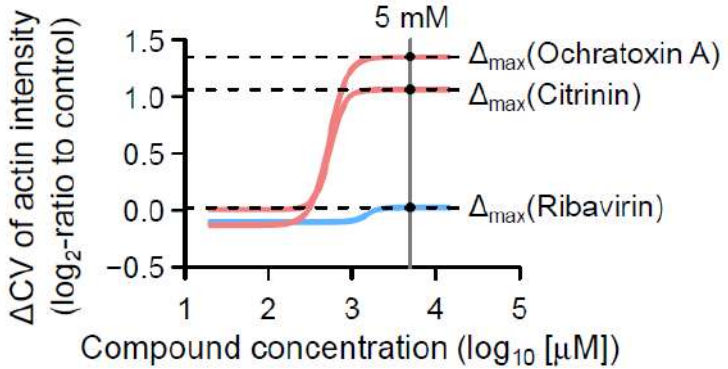
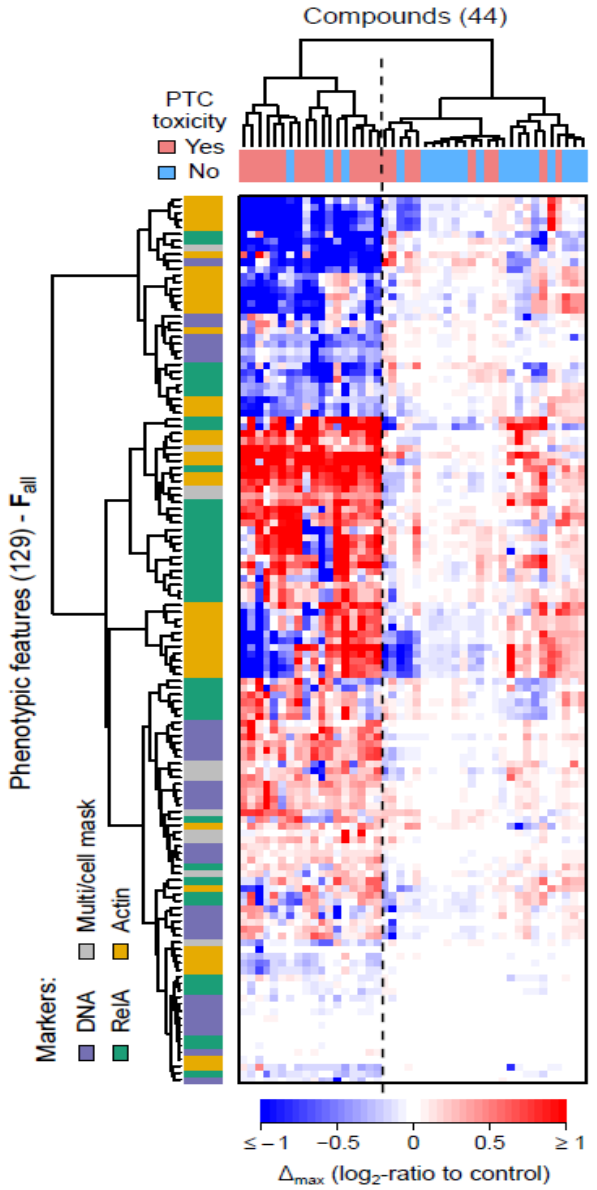
We performed phenotypic profiling … 129 features

Ran Su







Single-feature classifiers

$\mathbf{f}_1 \rightarrow$ Random forest $\rightarrow acc(\mathbf{f}_1)$

$\mathbf{f}_2 \rightarrow$ Random forest $\rightarrow acc(\mathbf{f}_2)$

$\mathbf{f}_{129} \rightarrow$ Random forest $\rightarrow acc(\mathbf{f}_{129})$

$\mathbf{f}_{best}$

Multi-feature classifiers

$\mathbf{F}_{all} = \{\mathbf{f}_1, \mathbf{f}_2, ..., \mathbf{f}_{129}\}$

Recursive feature elimination ↔ Random forest

$\mathbf{F}_s \subseteq \mathbf{F}_{all}$

Random forest → $acc(\mathbf{F}_s)$

5 features based on
DNA, actin, and γH2AX in HK-2
(89% accuracy)

[Su et al., Arch Tox, 2016]

CREATING GROWTH, ENHANCING LIVES

The first predictive and high-throughput in vitro model for nephrotoxicity

Ran Su is now an Associate Professor at Tianjin University, China

B12

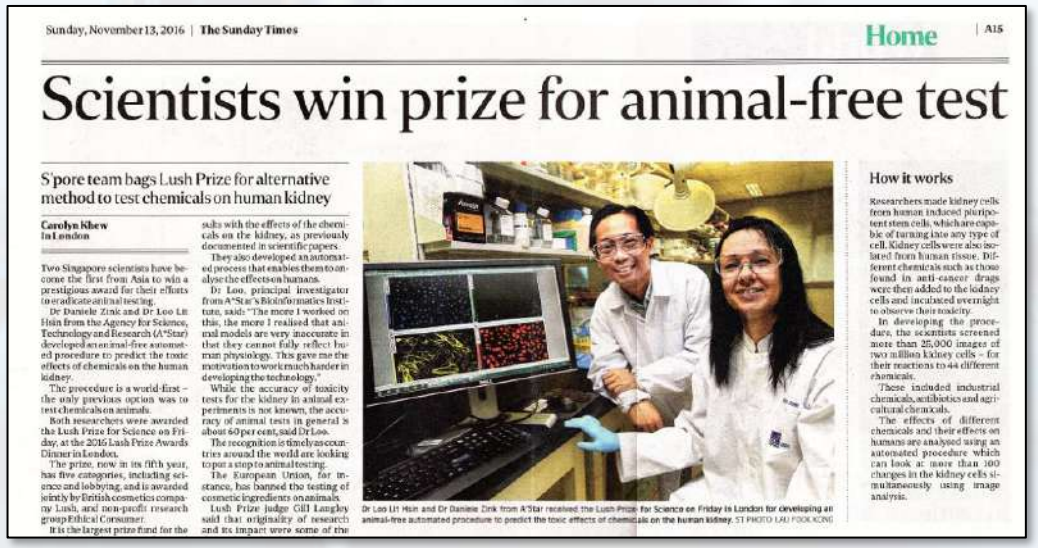
WILDLIFE SIGHTING MEET SINGAPORE'S LAST LEOPARD CATS B9

SCIENCE

Push for animal-free chemical safety tests

Singapore scientists will redouble efforts to develop animal-free methods for testing the safety of chemicals in new drugs and consumer products following a new research partnership between Singapore's Agency for Science, Technology and Research and the Environmental Protection Agency of the United States. Lin Yangchen looks at the potential of such work.

In 2016, Drs. Loo and Zink have become the first from Asia to win the Lush Prize (Science Award), UK



Sunday, November 13, 2016 | The Sunday Times

Home | A15

Scientists win prize for animal-free test

S'pore team bags Lush Prize for alternative method to test chemicals on human kidney

Carolyn Khew
In London

Two Singapore scientists have become the first from Asia to win a prestigious award for their efforts to eradicate animal testing.

Dr Daniele Zink and Dr Loo Lit Hsin from the Agency for Science, Technology and Research (A*Star) developed an animal-free automated procedure to predict the toxic effects of chemicals on the human kidney.

The procedure is a world-first – the only previous option was to test chemicals on animals.

Both researchers were awarded the Lush Prize for Science on Friday, at the 2016 Lush Prize Awards Dinner in London.

The prize, now in its fifth year, has five categories, including science and lobbying, and is awarded jointly by British cosmetics company Lush, and non-profit research group Ethical Consumer.

It is the largest prize fund for the tasks with the effects of the chemicals on the kidney, as previously documented in scientific papers.

They also developed an automated process that enables them to analyse the effects on humans.

Dr Loo, principal investigator from A*Star's Bioinformatics Institute, said: "The more I worked on this, the more I realised that animal models are very inaccurate in that they cannot fully reflect human physiology. This gave me the motivation to work much harder in developing the technology."

While the accuracy of toxicity tests for the kidney in animal experiments is not known, the accuracy of animal tests in general is about 60 per cent, said Dr Loo.

The recognition is timely as countries around the world are looking to put a stop to animal testing.

The European Union, for instance, has banned the testing of cosmetic ingredients on animals.

Lush Prize judge Gill Langley said that originality of research and its impact were some of the

Dr Loo Lit Hsin and Dr Daniele Zink from A*Star received the Lush Prize for Science on Friday in London for developing an animal-free automated procedure to predict the toxic effects of chemicals on the human kidney. ST PHOTO: LAU FOOK KONG

How it works

Researchers made kidney cells from human induced pluripotent stem cells, which are capable of turning into any type of cell. Kidney cells were also isolated from human tissue. Different chemicals such as those found in anti-cancer drugs were then added to the kidney cells and incubated overnight to observe their toxicity.

In developing the procedure, the scientists screened more than 25,000 images of two million kidney cells – for their reactions to 44 different chemicals.

These included industrial chemicals, antibiotics and agricultural chemicals.

The effects of different chemicals and their effects on humans are analysed using an automated procedure which can look at more than 100 changes in the kidney cells simultaneously using image analysis.

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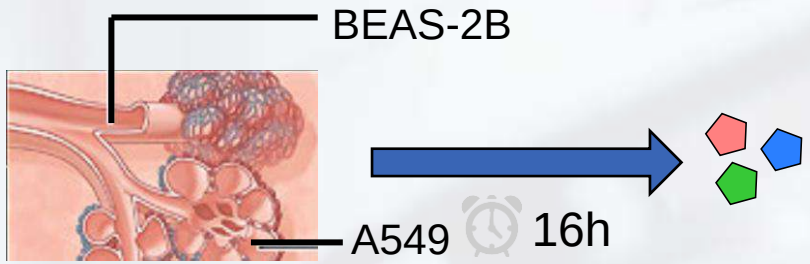
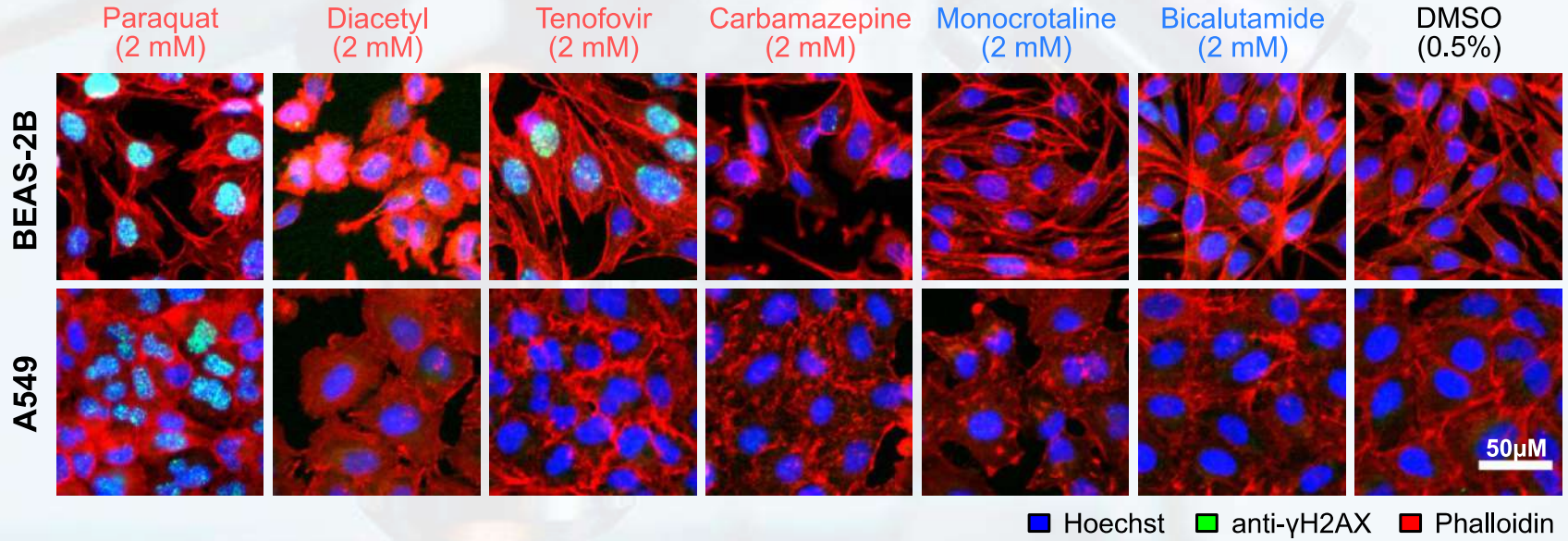
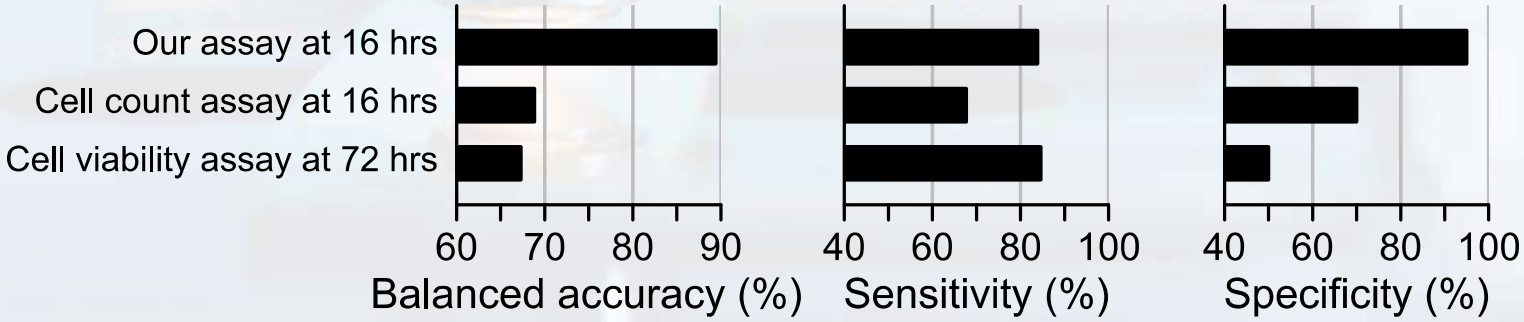
Building Lung Toxicity Model Using Phenotypic Profiling



HIPPTox lung assays are more accurate and specific than standard cell count or viability assays





Joey Lee

[Lee et al., Arch Tox, 2018]

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Archives of Toxicology (2020) 94:2749–2767
https://doi.org/10.1007/s00204-020-02778-3

IN VITRO SYSTEMS

Predicting direct hepatocyte toxicity in humans by combining high-throughput imaging of HepaRG cells and machine learning-based phenotypic profiling

Faezah Hussain[1] · Sreetama Basu[2] · Javen Jun Hao Heng[1] · Lit-Hsin Loo[2,3] · Daniele Zink[1]

Received: 12 November 2019 / Accepted: 5 May 2020 / Published online: 12 June 2020

Abstract

Accurate prediction of drug- and chemical-induced hepatotoxicity remains to be a problem for pharmaceutical companies as well as other industries and regulators. The goal of the current study was to develop an in vitro/in silico method for the rapid and accurate prediction of drug- and chemical-induced hepatocyte injury in humans. HepaRG cells were employed for high-throughput imaging in combination with phenotypic profiling. A reference set of 69 drugs and chemicals was screened at a range of 7 concentrations, and the cellular response values were used for training a supervised classifier and for determining assay performance by using tenfold cross-validation. The results showed that the best performing phenotypic features were related to nuclear translocation of RELA (RELA proto-oncogene, NF-kB subunit; also known as NF-kappa B p65), DNA organization, and the F-actin cytoskeleton. Using a subset of 30 phenotypic features, direct hepatocyte toxicity in humans could be predicted with a test sensitivity, specificity and balanced accuracy of 73%, 92%, and 83%, respectively. The method was applied to another set of 26 drugs and chemicals with unclear annotation and their hepatocyte toxicity in humans was predicted. The results also revealed that the identified discriminative phenotypic changes were related to cell death and cellular senescence. Whereas cell death-related endpoints are widely applied in in vitro toxicology, cellular senescence-related endpoints are not, although cellular senescence can be induced by various drugs and other small molecule compounds and plays an important role in liver injury and disease. These findings show how phenotypic profiling can reveal unexpected chemical-induced mechanisms in toxicology.

Example of how our models were used to assess fungicides

National Institute for Public Health and the Environment
Ministry of Health, Welfare and Sport

Chemical Research in Toxicology

pubs.acs.org/crt Article

A Case Study with Triazole Fungicides to Explore Practical Application of Next-Generation Hazard Assessment Methods for Human Health

Leo T. M. van der Ven,* Emiel Rorije, R. Corinne Sprong, Daniele Zink, Remco Derr, Giel Hendriks, Lit-Hsin Loo, and Mirjam Luijten

Cite This: *Chem. Res. Toxicol.* 2020, 33, 834–848 | Read Online

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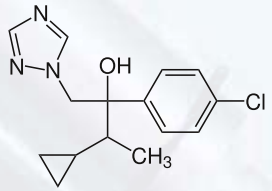
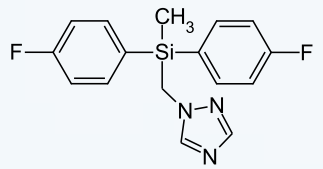
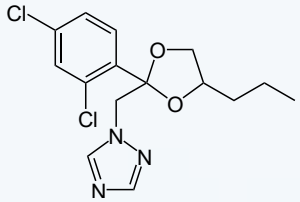
Three agricultural azole fungicides with unknown or unclear human toxicities



Cyproconazole



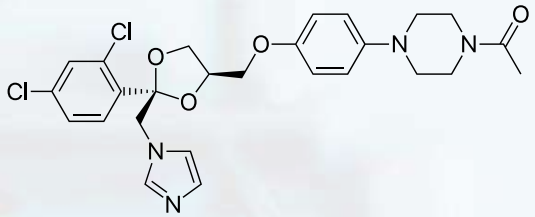
Flusilazole



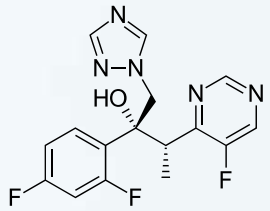
Propiconazole

What are their relative bioactivities?

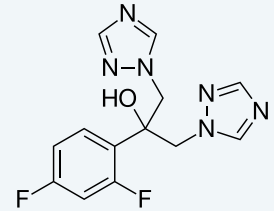
- To validate our model, we also add in 3 negative controls: azole drugs known to have low kidney effects



Ketoconazole



Voriconazole



Fluconazole

[van der Ven et al., Chem Res Tox, 2020]

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Our ranking of the chemicals agrees with predictions based on other toxicological endpoints made by RIVM

National Institute for Public Health and the Environment
Ministry of Health, Welfare and Sport

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Table 4. Potency Comparison[a]

Toxicological domain	Prediction Models (*n*)	Targeted endpoints in prediction models	Flu	Pro	Cyp	Ref
Cell functions	4	Dynamic cell state, mitochondrial function, metabolism, transporter functions	+	±	±	26-28
Endocrine perturbation, Mode of action screening	5	Endocrine profiling, nuclear receptors, G-protein-coupled receptors (aminergic/other)	±	±	±	26, 29
Endocrine perturbation, specific	8	Estrogen signalling pathway, estrogen receptor interaction, androgen receptor interaction, aromatase inhibition, steroidogenesis, thyroid hormone synthesis	++	+	+	30-37
Metabolic disorder	7	Glucose metabolism, adipocyte function, feeding behaviour	+	+	±	38, 39
Developmental toxicity	8	Developmental toxicity in rats, rabbits, zebrafish, *C.elegans*; vascular development	++	++	+	40-46
(Developmental) Neurotoxicity	6	Ion channel, transporters, enzymes, neuronal network activity, neurobehavior	+	+	±	26, 47-49
Hepatotoxicity	2	Hypertrophy, liver injury, proliferative lesions, oxidative stress	+++	++	+	50, 51
Nephrotoxicity	1	Renal proximal tubular cell toxicity	+++	+++	+	17
Genotoxicity	5	DNA damage, gene mutations, chromosomal aberrations, p53 activation and oxidative stress	-	-	-	9-11, 14, 15, 19, 20, 52, 53
Carcinogenicity	2	Nuclear receptor activity, cancer hazard prioritization (hallmark genes)	+	++	+	54, 55
LOAEL embryotoxicity			10	35	20	
LOAEL hepatotoxicity			2.4	121	25.3	
LOAEL carcinogenicity			384	108	13.2	
Acceptable Daily Intake (ADI)			0.007	0.07	0.02	

[van der Ven et al., Chem Res Tox, 2020]

James Miller

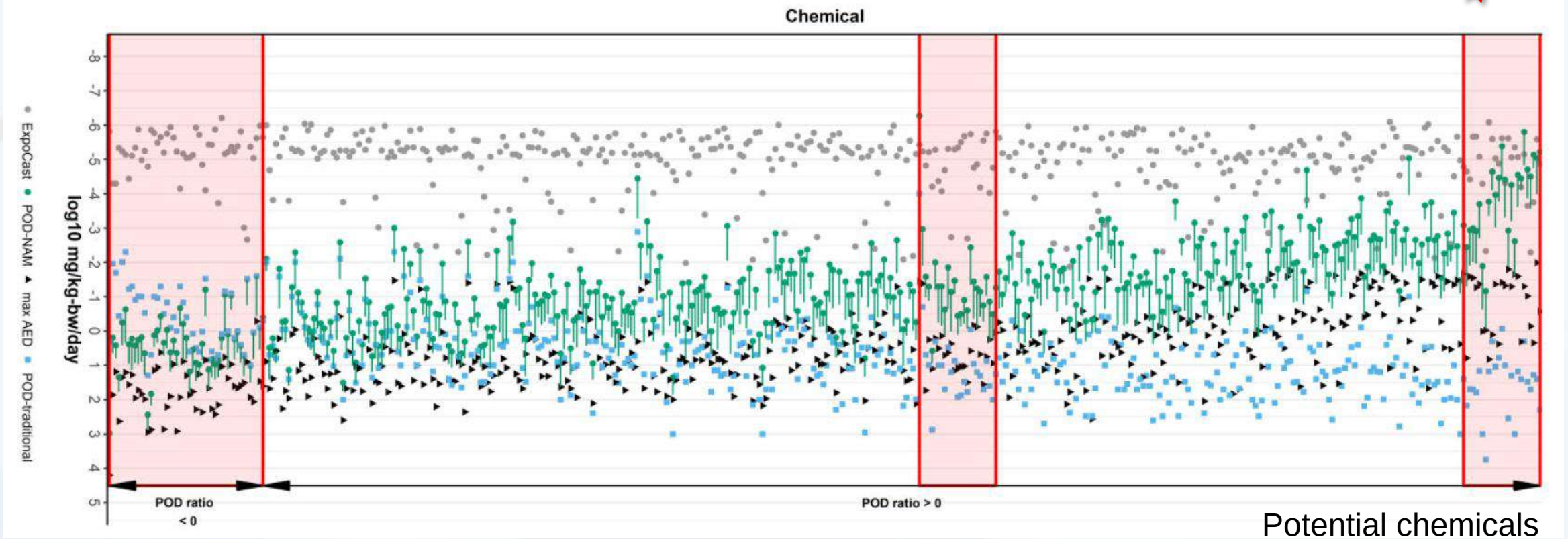
APCRA
ACCELERATING THE PACE OF CHEMICAL RISK ASSESSMENT

CREATING GROWTH, ENHANCING LIVES

International case study on the use of in vitro bioactivity in risk-based chemical prioritization

[Paul-Friedman et al., Tox Sci, 2020]

SOT Paper of the Year Award (Honorable Mention), 2020



Potential chemicals of concern

- Predicted exposure level based on modeling
- PODs based on NAMs (ToxCast + HIPPTox)
- PODs based on traditional animal models

United States Environmental Protection Agency
A*STAR
Health Canada
ECHA EUROPEAN CHEMICALS AGENCY
efsa European Food Safety Authority

ToxMAD

Toxicity Mode-of-Action Discovery Platform

Current safety assessments

Phenotypic endpoints

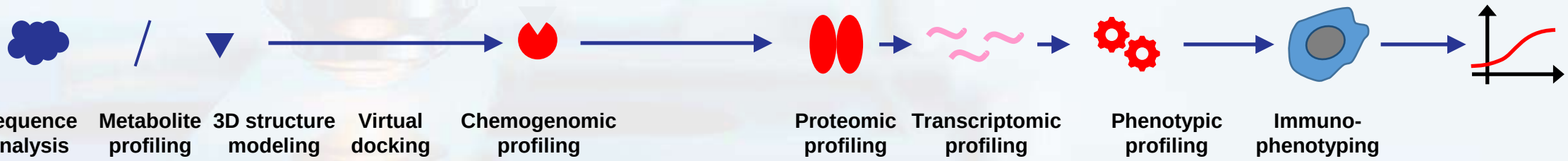
Future safety assessments

Mechanistic reasoning

ToxMAD is based on various A*STAR-developed *in vitro* and *in silico* technologies, and aim to rapidly and efficiently identify MoAs of chemicals (especially **key molecular initiating events and cellular events** leading to adverse outcomes)

Molecular initiating events

Key molecular/cellular events



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A platform to publish and share large biological images

How do tumors with poor outcomes look like?

Is my staining correct?

Does this patient have similar phenotypes as the published cases?

How do I train or benchmark my AI algorithms?

- Internet search engines
- Journal websites or online data repositories
- Online forums or social media sites
- Bioimage databases
- Suppliers' product websites
- Colleagues

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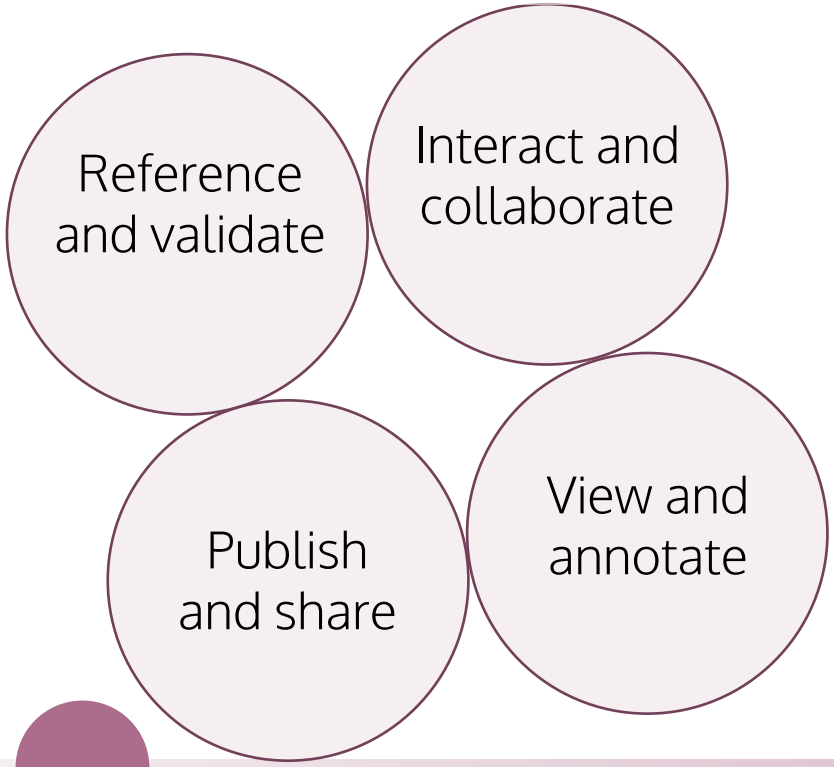
Introducing ImmunoAtlas ...

Standard or reference images of clinically-relevant immunofluorescence or immunotherapy markers
(Users include researchers, pathologists, standard workgroup, and companies)



ImmunoAtlas

Bioimage publishing and sharing portal (2021)

HistoPath Analytics (HPA) Platform
Online tissue image management and analysis platform (2019-2021)

cellXpress 2.0
Image processing engine in C++ (2011-2021)

Bioinformatics Institute
BII

Institute of Molecular and Cell Biology
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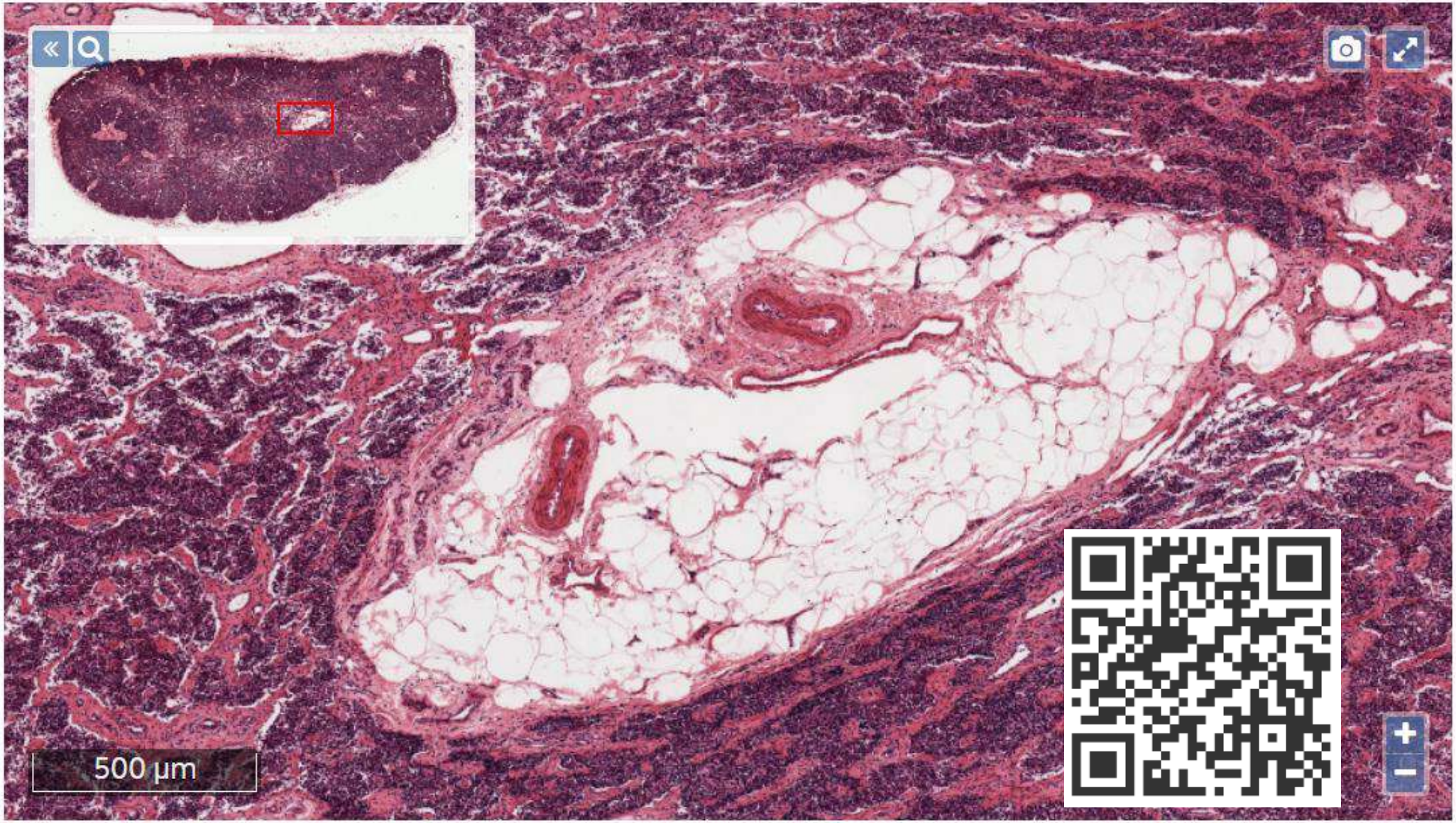
Singapore General Hospital
SingHealth

CREATING GROWTH, ENHANCING LIVES

Image format Aperio SVS Dimensions 11.55 mm)

Samples collected for the case:



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