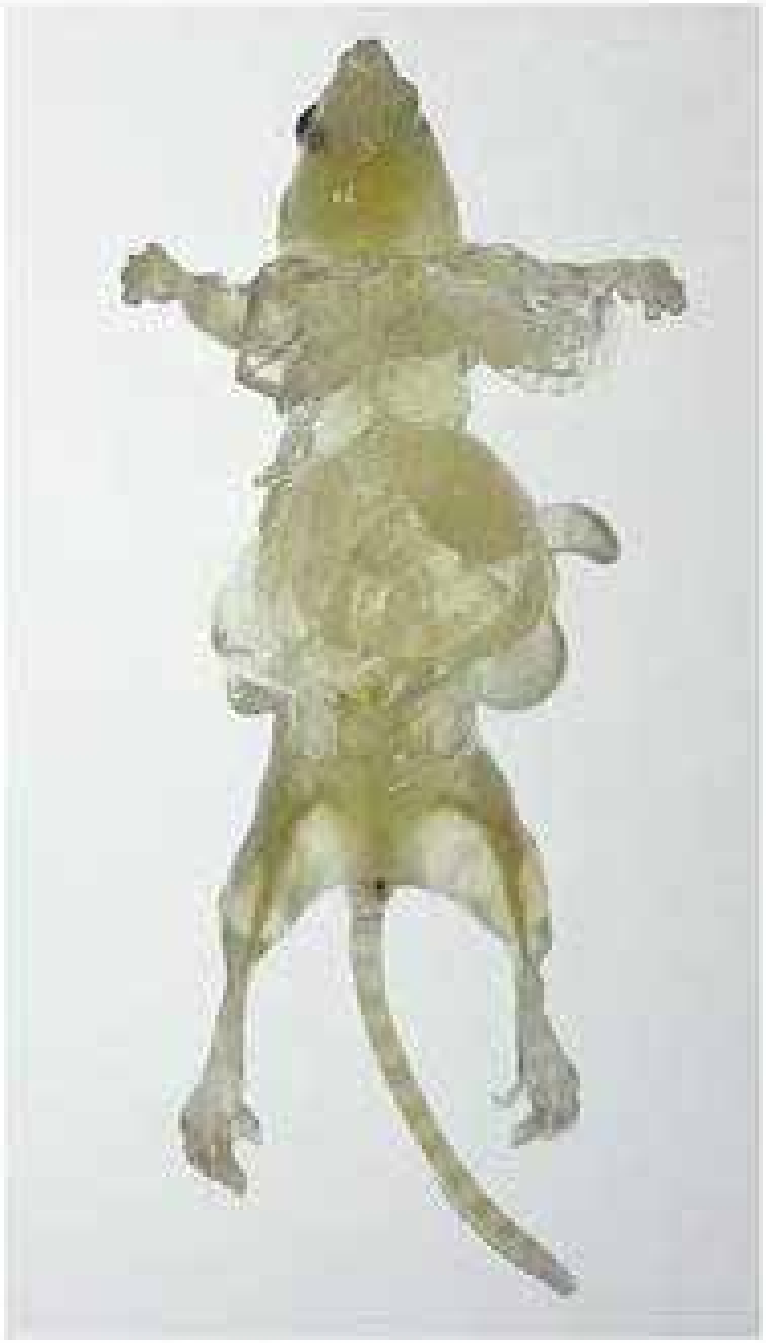
全身透明化の先に見えるもの

上田泰己
東京大学医学系研究科
理化学研究所

ジョルジュ・スーラ「グランド・ジャット島の日曜日の午後」1886 シカゴ美術館、アメリカ

全身の細胞数

どのように解読するのか？



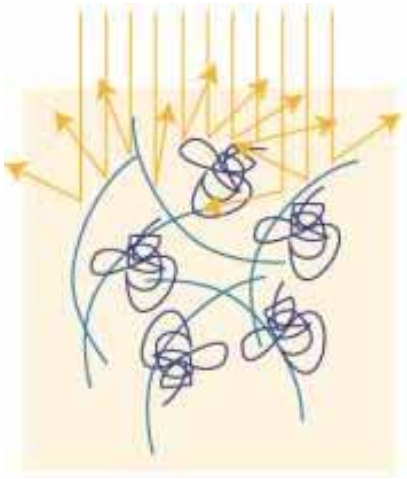
全身細胞解析

5000 um

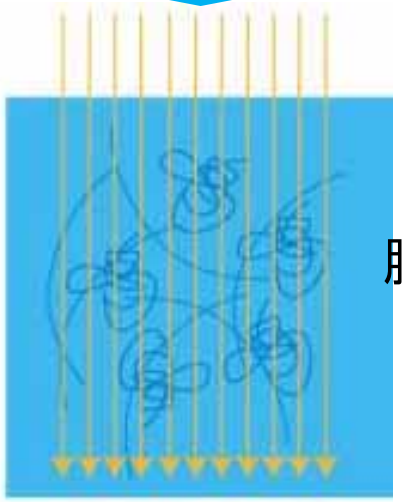
組織透明化の原理

①これまでの先行研究は光の散乱に注目

光の散乱



生体組織には
屈折率の異なる
様々な物質が存在

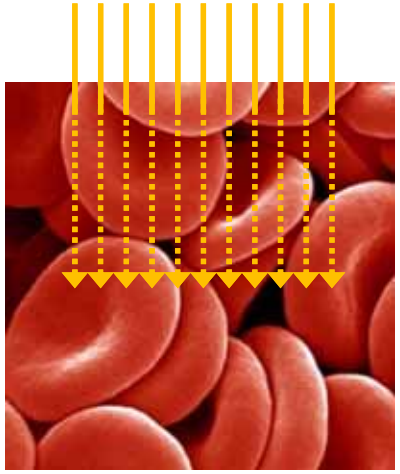


脂質除去・水置換等
により各物質間の
屈折率差を調整

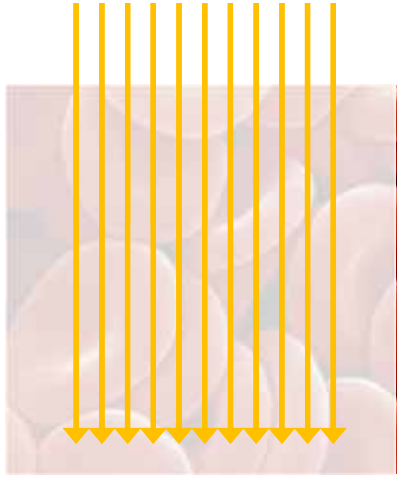
屈折率を調整することで
光の散乱を抑制し透明化を促進

②光の吸収を抑制するベストな方法はなかった

光の吸収



生体組織には
光を吸収する色素
（例：ヘム）が存在



色素除去により
光の吸収を抑制

生体色素を除去することで
光の吸収を抑制し透明化を促進

透明化研究の先駆者

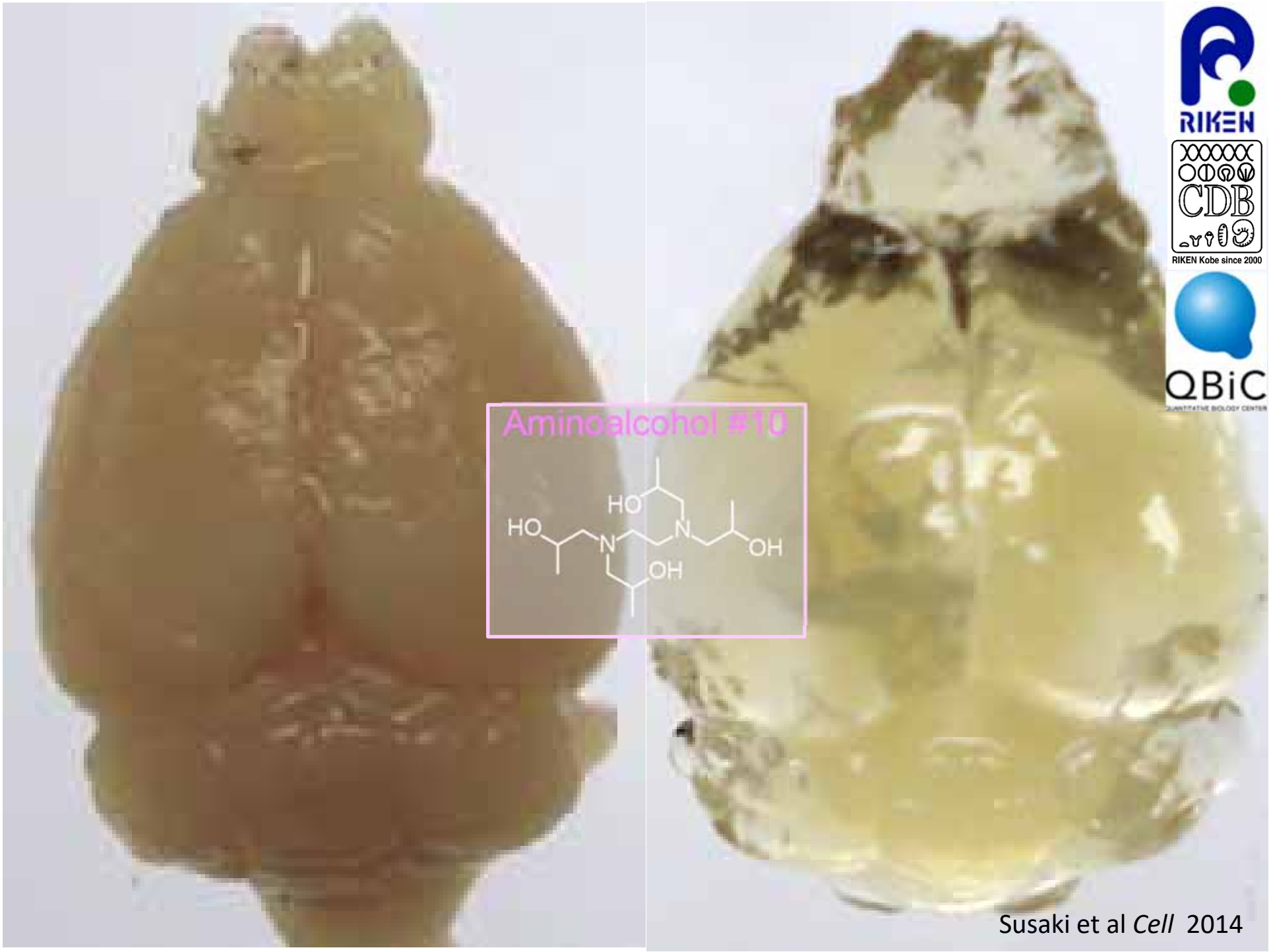
疎水性試薬
(1911~)

Werner Spalteholz
(1861-1940, Germany)

親水性試薬
(1995~)

Valery V. Tuchin
(1944-, Russia)

Fumiaki Kishino



RIKEN

CDB

RIKEN Kobe since 2000

QBiC

QUANTITATIVE BIOLOGY CENTER

Susaki et al *Cell* 2014

Masamitsu Iino

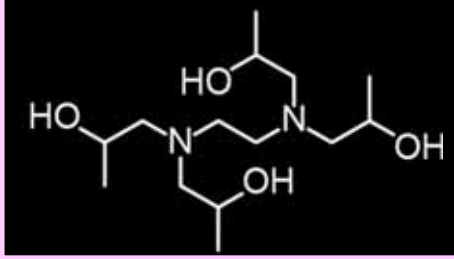
Kazuki Tainaka

Shimpei Kubota

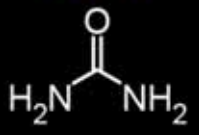
http://pulpbits.net/7-pictures-of-red-blood-cells/red-blood-cells-picture/

Kazuki Tainaka
(Chemistry)

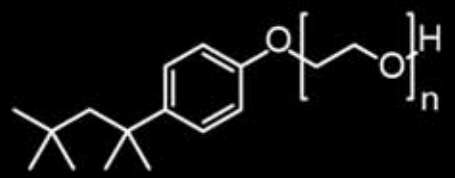
Aminoalcohol #10



Urea

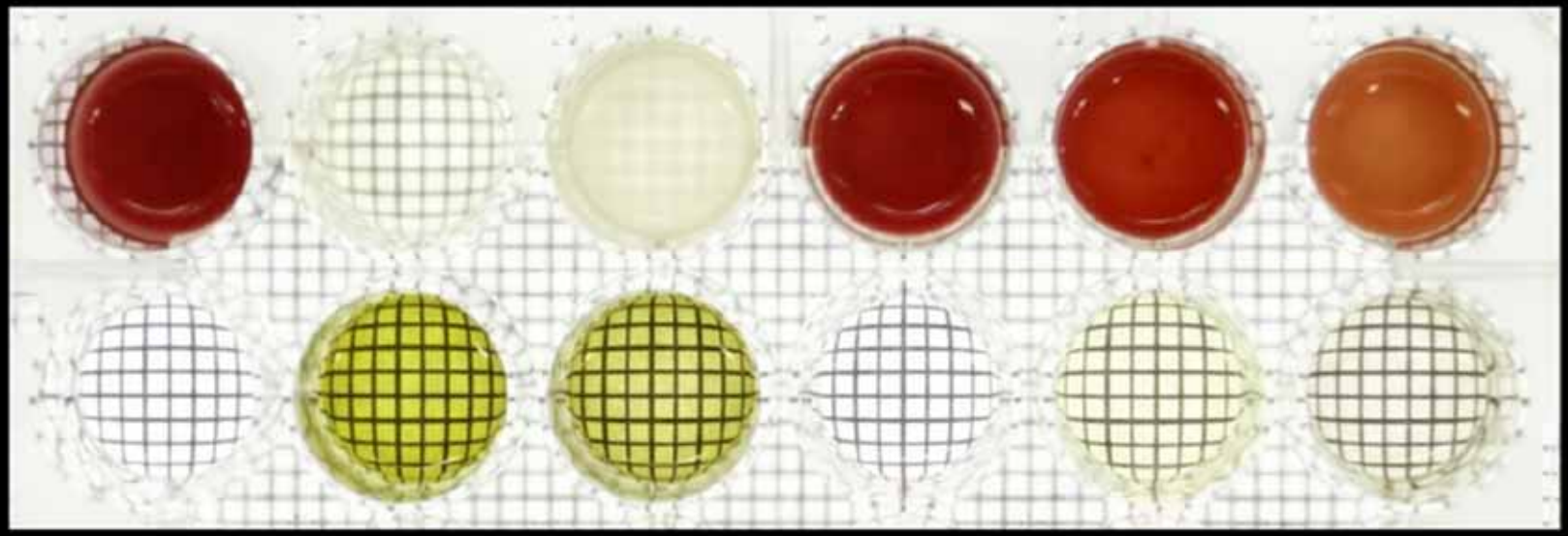


Triton X-100



PBS
CUBIC-1
#10
Urea
Triton X
NaOH

Supernatant Pellet



Shimpei Kubota
(Medicine)

全身･各臓器の透明化

各臓器の透明化

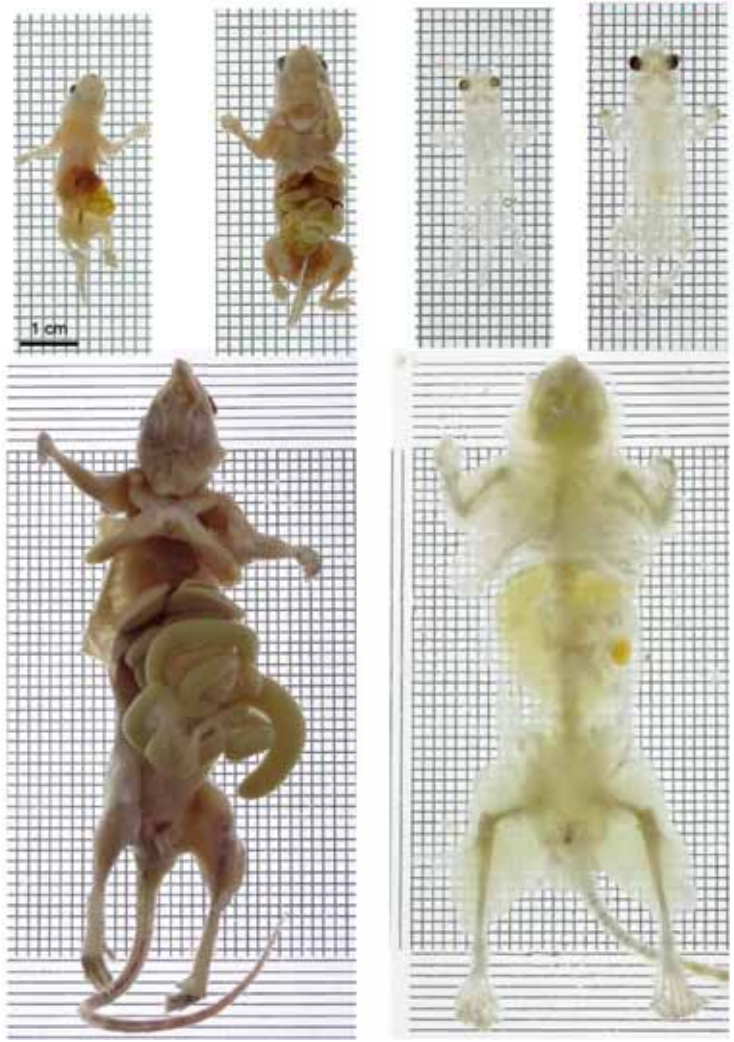
透明化処理前

透明化処理後

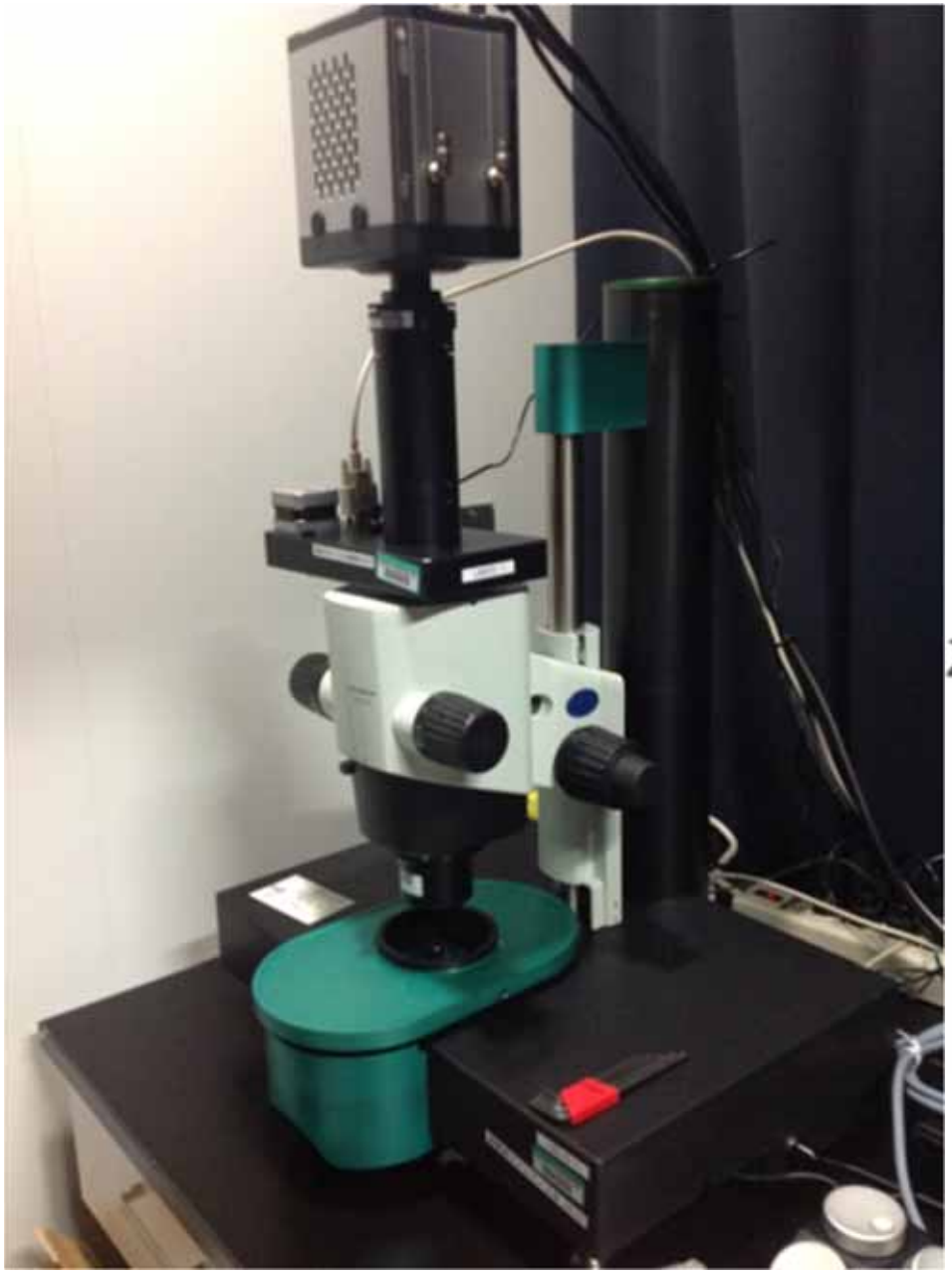
全身の透明化

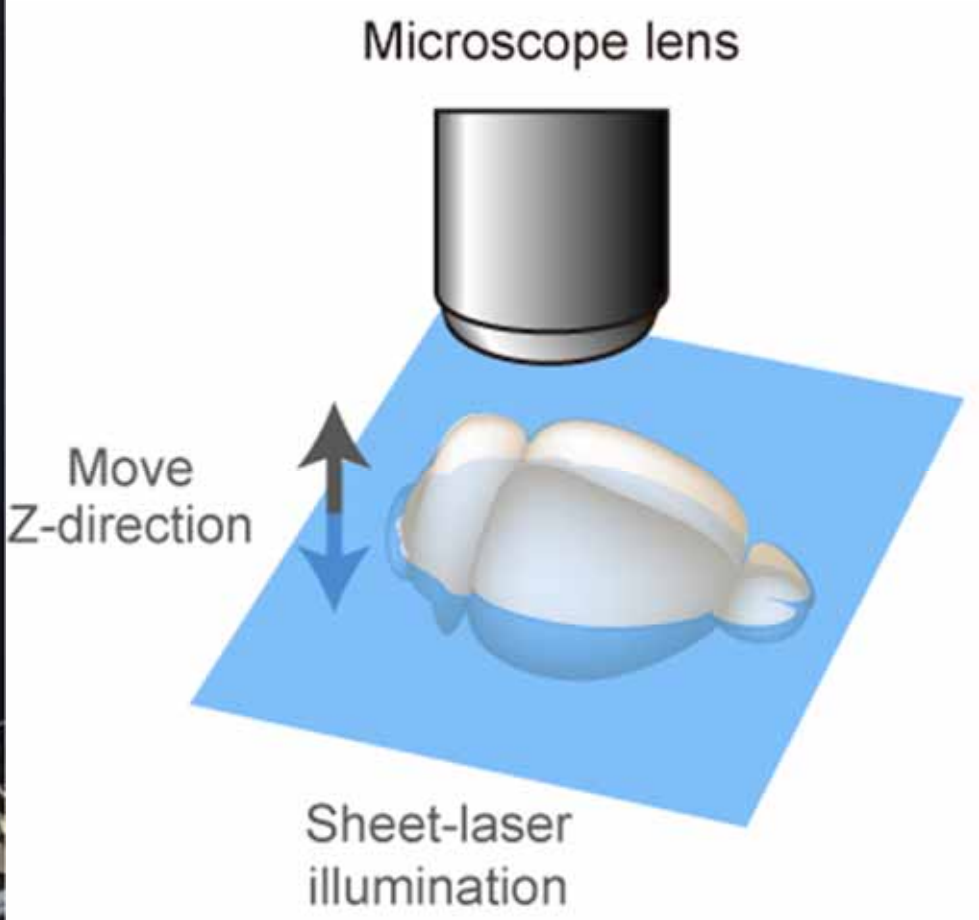
透明化処理前

透明化処理後



光シート顕微鏡




Microscope lens
Move
Z-direction
Sheet-laser
illumination

3次元解剖学：各種臓器の3次元ー細胞解像度イメージ

全身透明化技術の国内外での反響①

nature

Images of the year 2014

nature methods
Techniques for life scientists and chemists

Methods of the year 2014
(“Methods to Watch”)

nature International weekly journal of science

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Archive > Volume 516 > Issue 7531 > News > Article

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NATURE | NEWS

365 days: Images of the year

Eruptions, comets and a see-through mouse all captured the imagination in 2014.

Daniel Cressey

17 December 2014

PDF Rights & Permissions

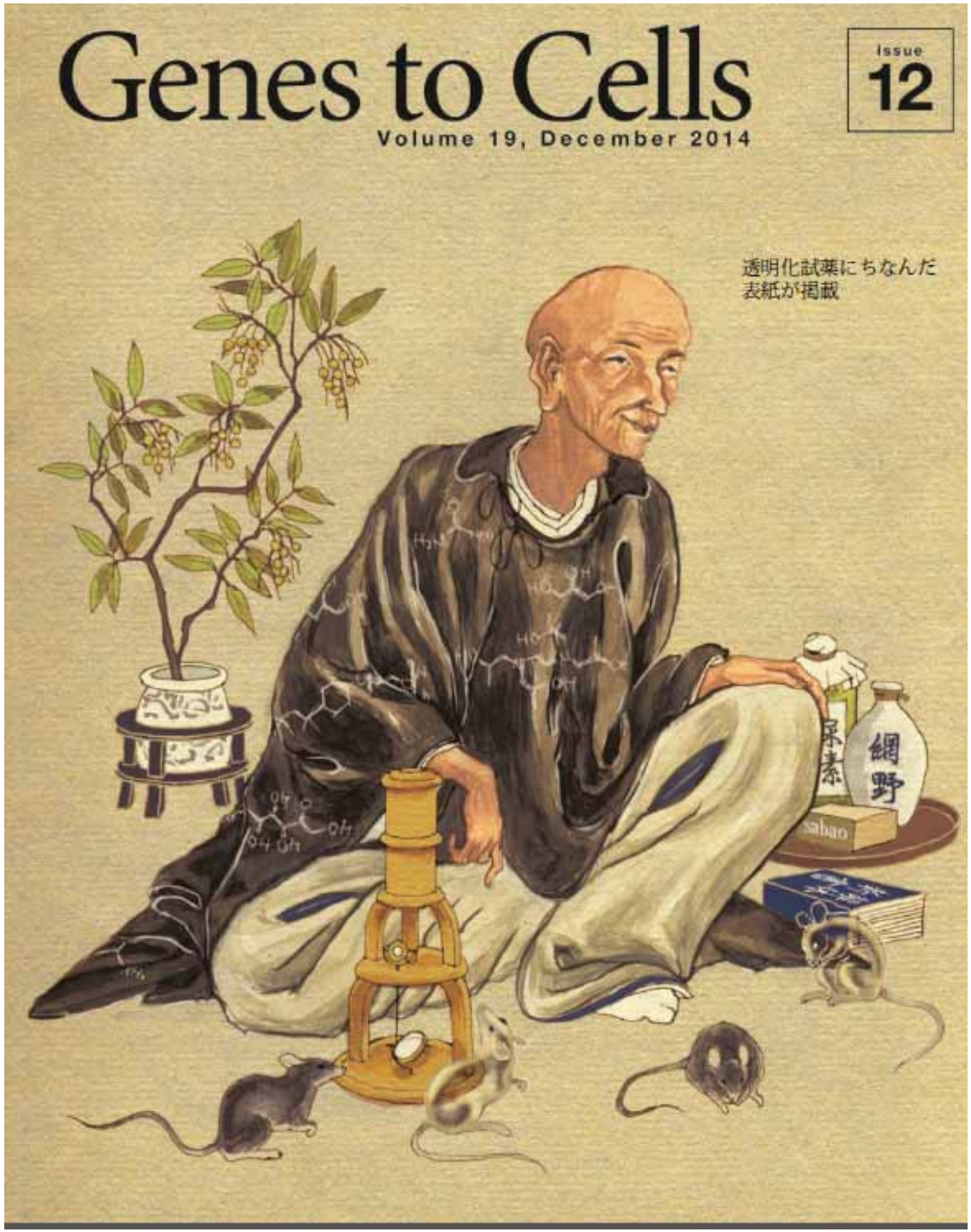
Incredible discoveries in 2014 arose from researchers' relentless pursuit of answers about the

Special

全身透明化技術の国内外での反響②

THE WALL STREET JOURNAL.	Japanese Scientists Make ‘Nearly-Invisible’ Mouse (Nov 7, 2014)
The Washington Post	Researchers in Japan create creepy-looking transparent mouse (Nov 7, 2014)
The Japan Times	Japanese scientists succeed in making mice transparent (Nov 7, 2014)
THE HUFFINGTON POST	Transparent Nearly Invisible Mice Created By Japanese Scientists (Nov 11, 2014)
INTERNATIONAL BUSINESS TIMES	Japanese Scientists Create 'Transparent' Mice (Nov 7, 2014)
DAILY NEWS	Japanese scientists turn mouse ‘nearly invisible’ (Nov 9, 2014)
UPI. com	Researchers unveil see-through mouse (Nov 7, 2014)
Headlines & Global News	Invisible Mouse Created By Japanese Scientists (Nov 11, 2014)
Science Daily	Images of a nearly invisible Mouse (Nov 6, 2014)
Mail Online	Scientists create ‘see-through’ mice that could help show how cancer develops (Nov 6, 2014, 英紙)
Sciences et Avenir	Incroyable:des chercheurs japonais créent une souris transparente（仏紙, Nov 7, 2014）
Galileonet	E il topo diventa trasparente（伊紙, Nov 10, 2014）

朝日新聞（11月7日）
読売新聞（11月7日、24日）
日本経済新聞（11月7日）
毎日新聞（11月27日）
東京新聞（11月7日）
化学工業日報（11月10日、26日）
中日新聞（11月7日）
河北新報（11月7日）
日経産業新聞（11月7日）
日刊工業新聞（11月7日）
原子力産業新聞（11月20日）
科学新聞（11月14日）
サイエンスポータル（11月7日）



CUBIC技術が
杉田玄白先生と登場
「解体新書（1774年）」

CUBIC

- Aminoalcohols (「網野」)
- Urea(「尿素」)
- 界面活性剤(「sabao」)

New

TCI

Animal Tissue-Clearing Reagent CUBIC

Advantages

- Whole-body clearing is achieved using two reagents, T3740 CUBIC-L (for delipidation and decoloring) and T3741 CUBIC-R+ (for RI matching).
- The quenching of fluorescence signal is low.
- The period of sample treatment is shorter.
- The combination with light-sheet fluorescent microscopy (LSFM) or confocal laser-scanning microscopy (CLSM) enables the whole-organ / body imaging at a cellular resolution.

These products were developed by Prof. Hiroki R. Ueda (The University of Tokyo / RIKEN) and are under invention licenses by RIKEN, Japan.

Direction for Use : Mouse whole-body clearing protocol

Whole-body clearing

Whole-body clearing with propidium iodide staining

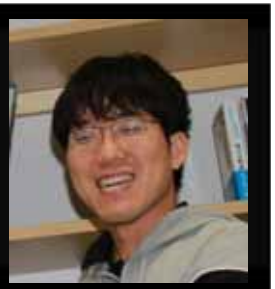
Pre-treatment	Delipidation	Wash x 3	Pre-treatment	RI match
50% CUBIC-L > 6 hr	CUBIC-L > 5 days	PBS > 2hr x 3	50% CUBIC-R+ 1 day	CUBIC-R+ > 1 day

Process	Reagent	Temp.	Time	Notes
Perfusion fixation	PBS			Finally, the mouse should be perfused with 50% CUBIC-L which is a 1:1 mixture of water and CUBIC-L.
	4% PFA in PBS			
Perfusion	PBS			
	50% CUBIC-L			
Pre-treatment	50% CUBIC-L	37℃	> 6 hr	Immerse the whole body of the mouse with gentle shaking (same in following steps). This step can be omitted.
Delipidation	CUBIC-L	37℃	> 5 days	Refresh CUBIC-L on day 1, day 2 and every 2 days after day 4
Wash x 3	PBS	RT	> 2hr x 3	Total 1 day
Pre-treatment	50% CUBIC-R+	RT	1 day	1:1 mixture of water and CUBIC-R+
RI matching	CUBIC-R+	RT	> 1 day	

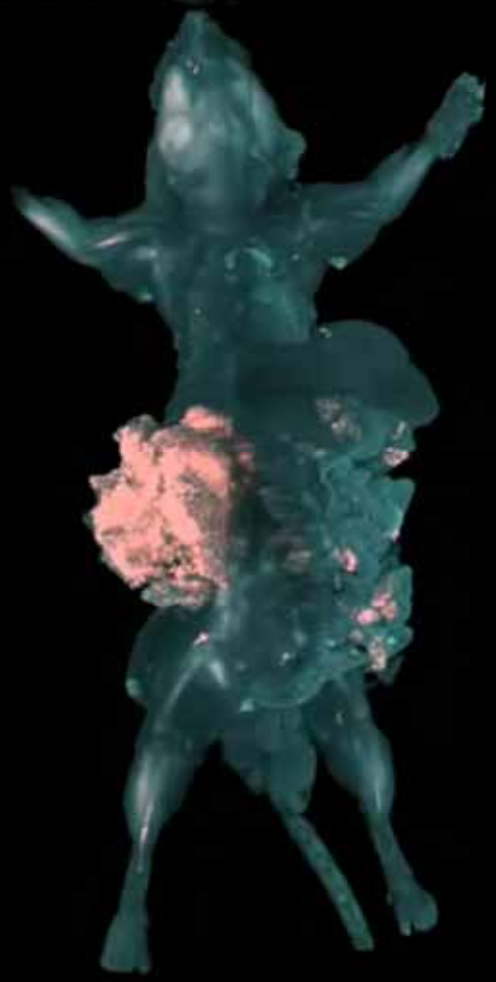
Work samples in a tube in which whole-body can be contained.
PFA : paraformaldehyde, RT : room temperature

全身透明化によるがん転移の観察

がん細胞　細胞核

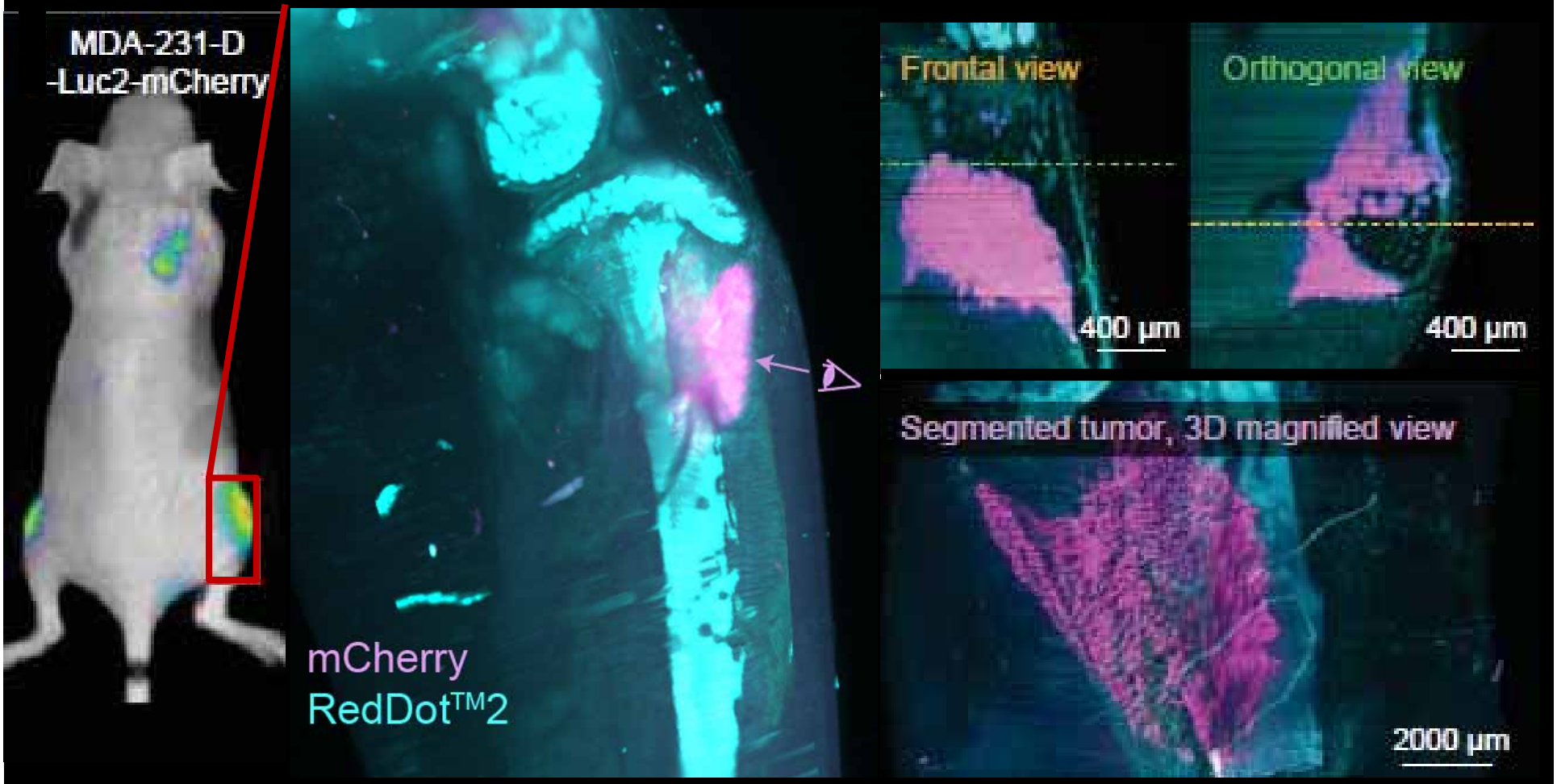


Shimpei Kubota
(医学)

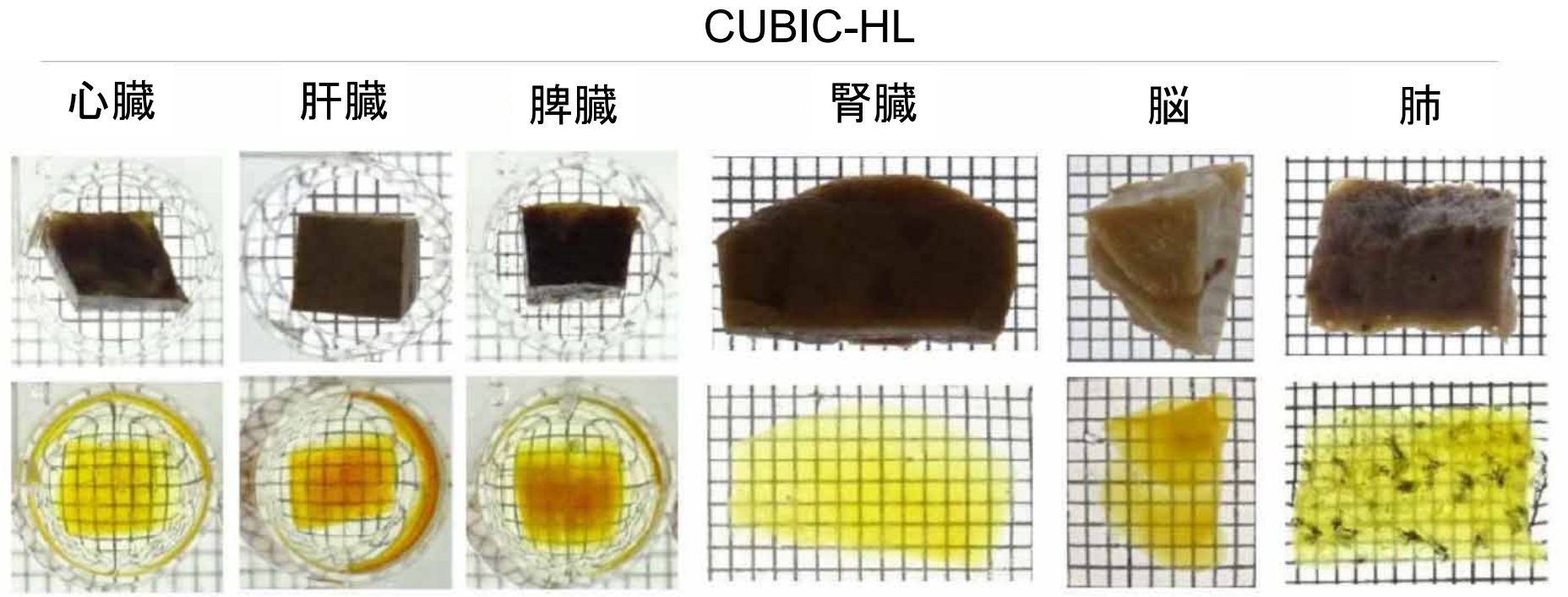


Kubota, Takahashi et al *Cell Reports*, 2017

CUBIC-Bを用いた骨の透明化（がんの骨転移）



CUBIC-HLを用いたヒト臓器の透明化



Tainaka, Murakami et al, in press

改良CUBICによる甲殻類の透明化（Konnoら）

ダンゴムシ

CUBIC

before

after

改良CUBIC

before

after

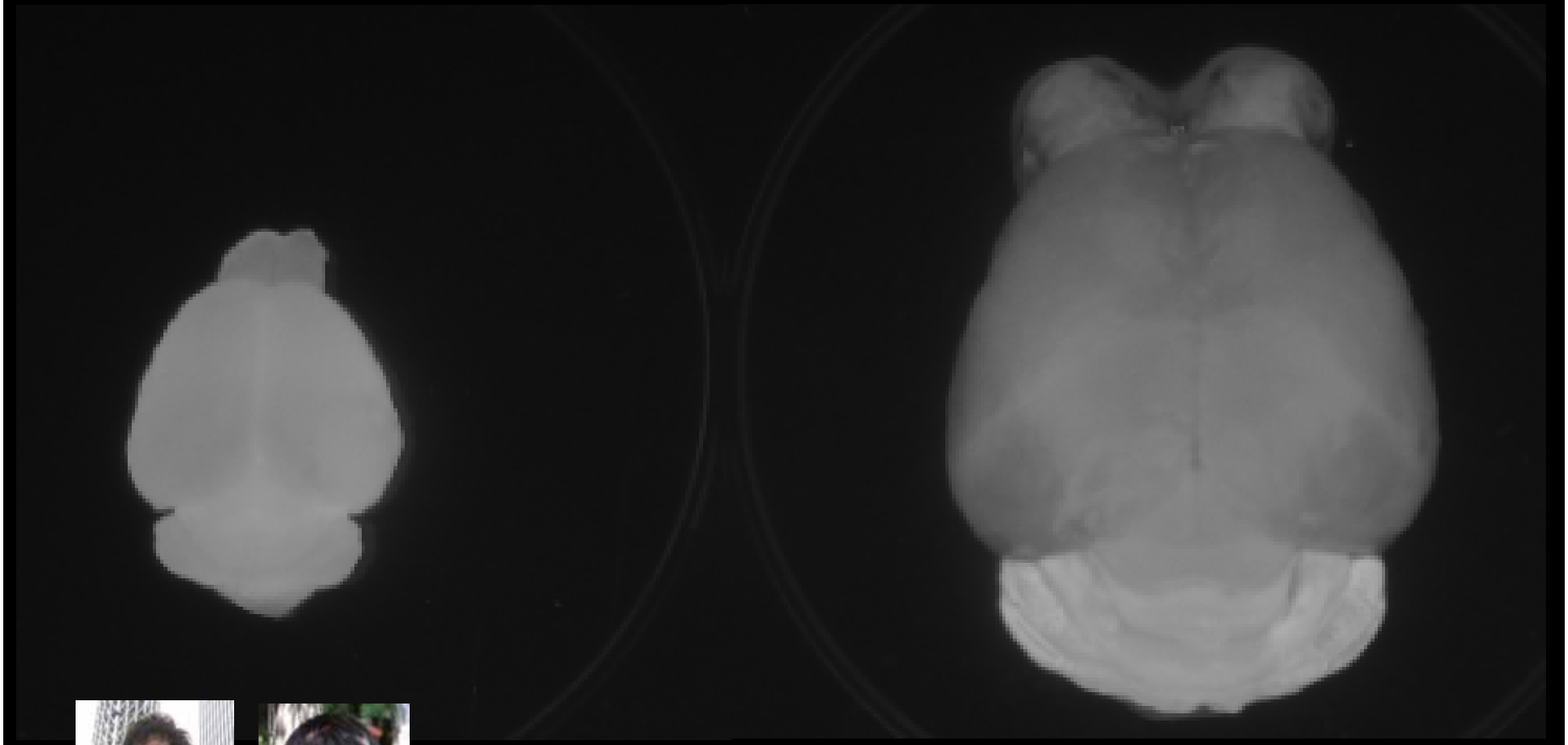
カニ

before

after

Konno & Okazaki. ***Zoological Letters*** 2018 4:13

CUBIC-Xを用い脳を体積比10倍以上に脳を膨潤



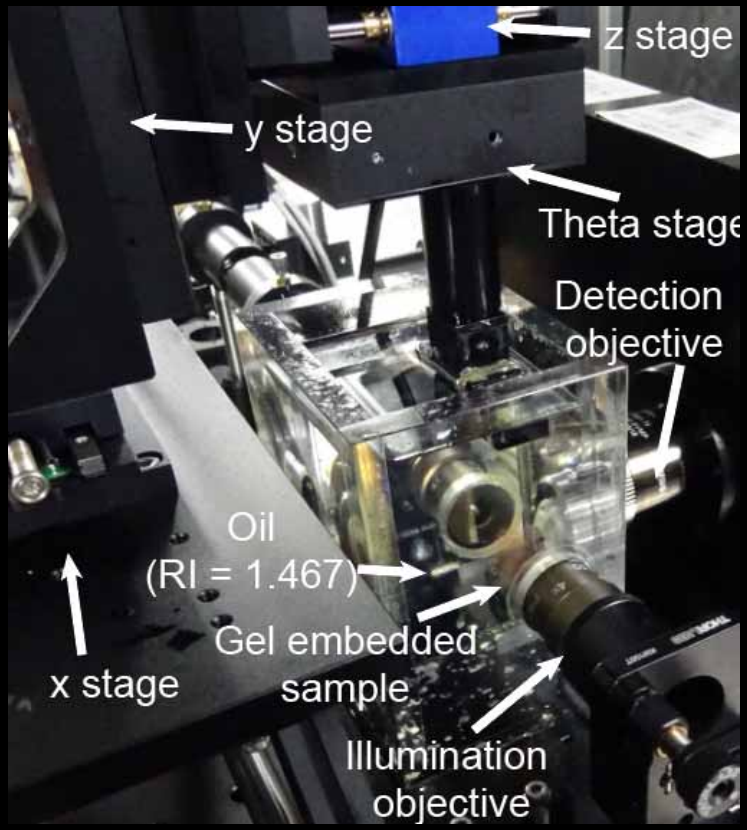
Tatsuya C. Murakami Tomoyuki Mano

Murakami et al, Nature Neuroscience, 2018

Advanced Light Sheet Microscope with High-Speed and High-Resolution

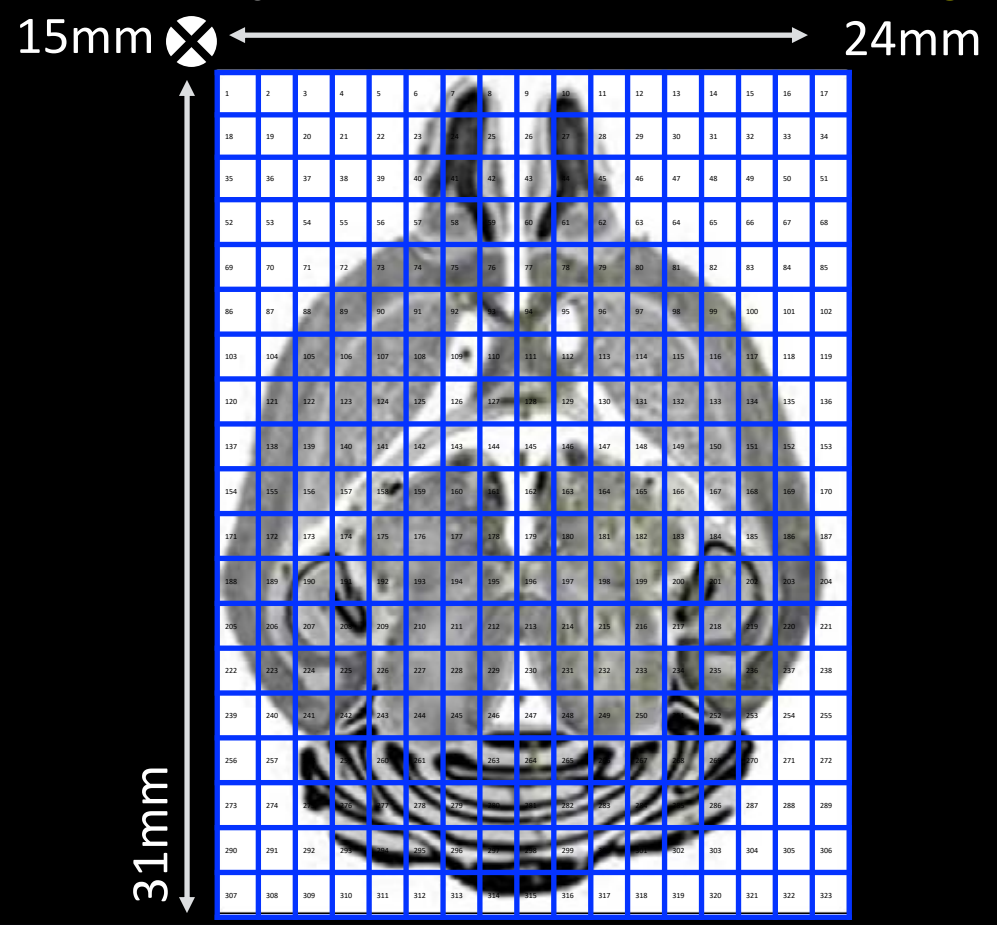
High resolution

~ NA = 0.6 objective detection lens
~ WD = 8 mm
-> High resolution (**0.5 um**) imaging



High speed & Large Data Size

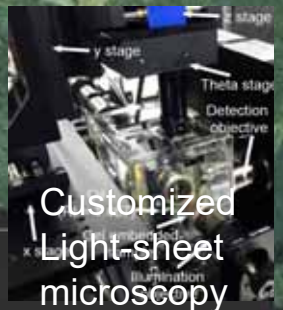
~ 5 images/sec (1,000 images within 3 min)
18~21 tile x 17~20 tile (~400 stack)
~3200 images x ~400 stack = **~1.3 million images**



Murakami et al, Nature Neuroscience, 2018

CUBIC-Xを用いた全脳全細胞解析（世界初）

suya C. Murakami Tomoyuki Mano

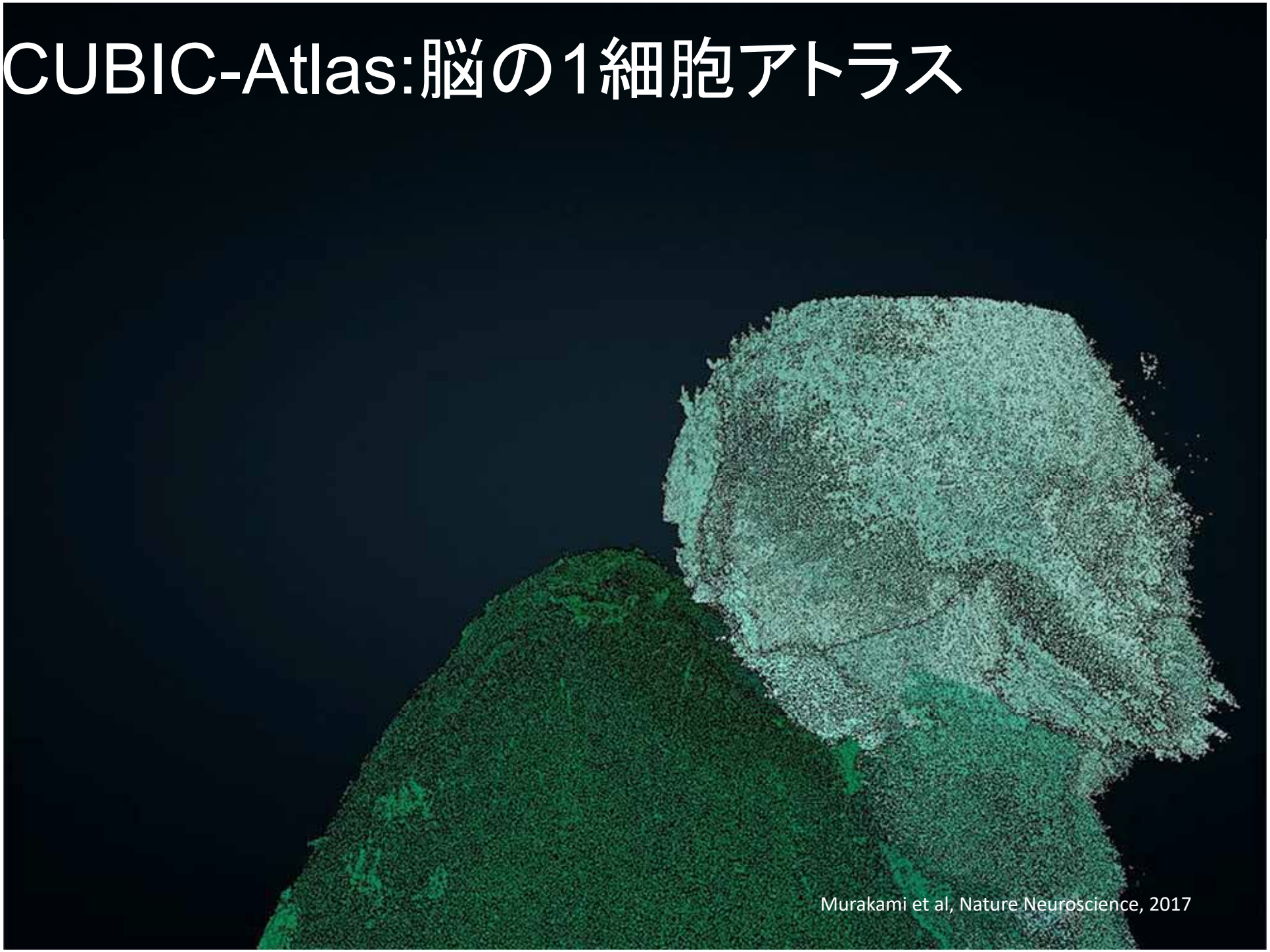


Customized Light-sheet microscopy

The movie is made by Arivis

Murakami et al, Nature Neuroscience, 2018

CUBIC-Atlas:脳の1細胞アトラス

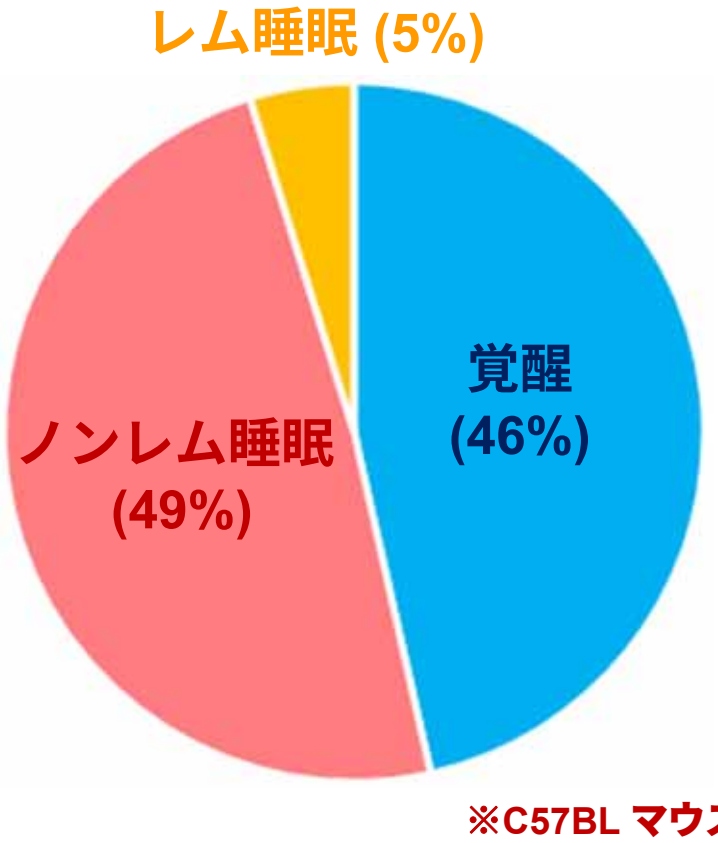


Murakami et al, Nature Neuroscience, 2017

脳の3つの状態

神経細胞

脳

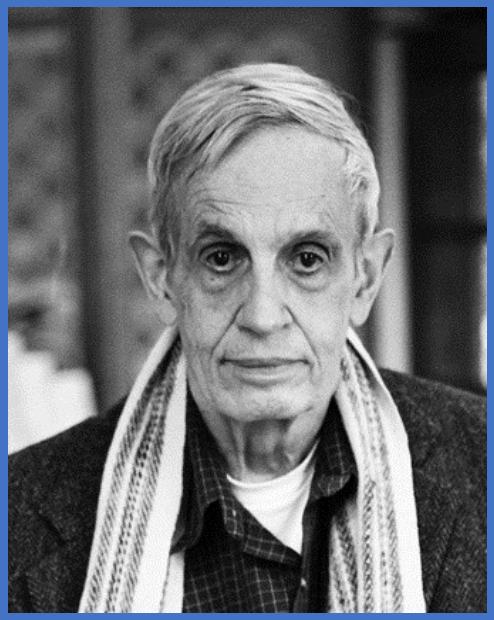


※C57BL マウス

脳の3つの状態の理解は、難問（意識・知性・自己）の基盤になる。

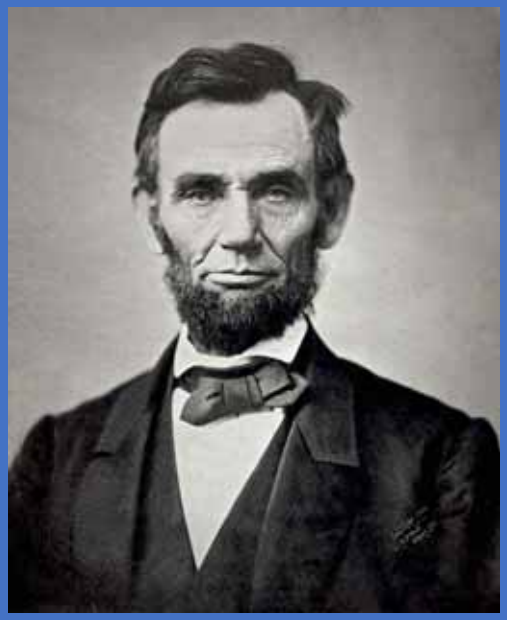
睡眠研究の重要性

統合失調症



John Forbes Nash, Jr
(1928-2015)

うつ病/
双極性障害



Abraham Lincoln
(1809-1865)

パーキンソン病

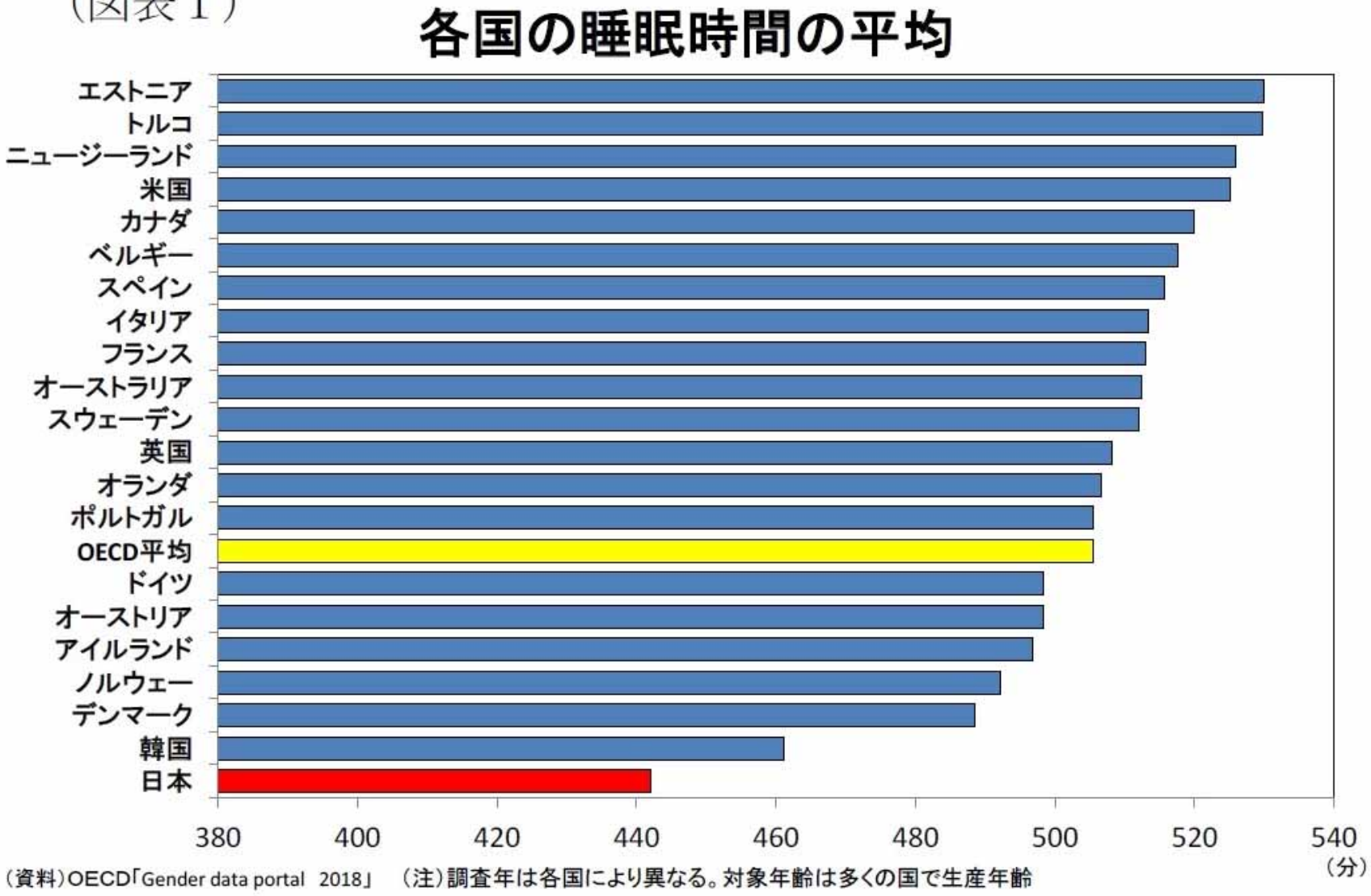
Michael J. Fox
(1961-)

https://en.wikipedia.org/wiki

多くの精神疾患・神経変性疾患は
睡眠障害を併発する

睡眠負債大国（日本）

（図表１）



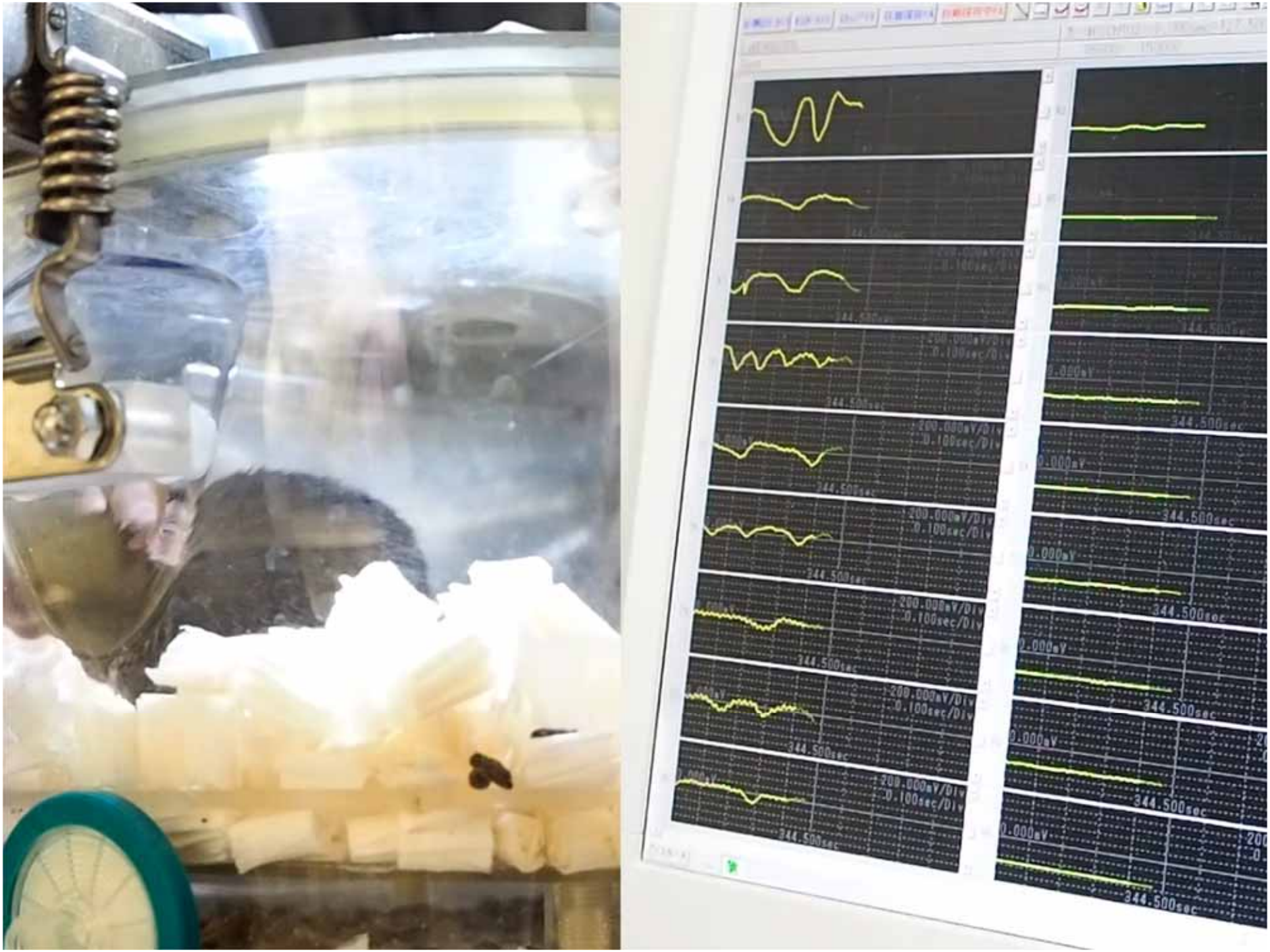
（資料）OECD「Gender data portal　2018」　（注）調査年は各国により異なる。対象年齢は多くの国で生産年齢

マウス睡眠研究@理化学研究所

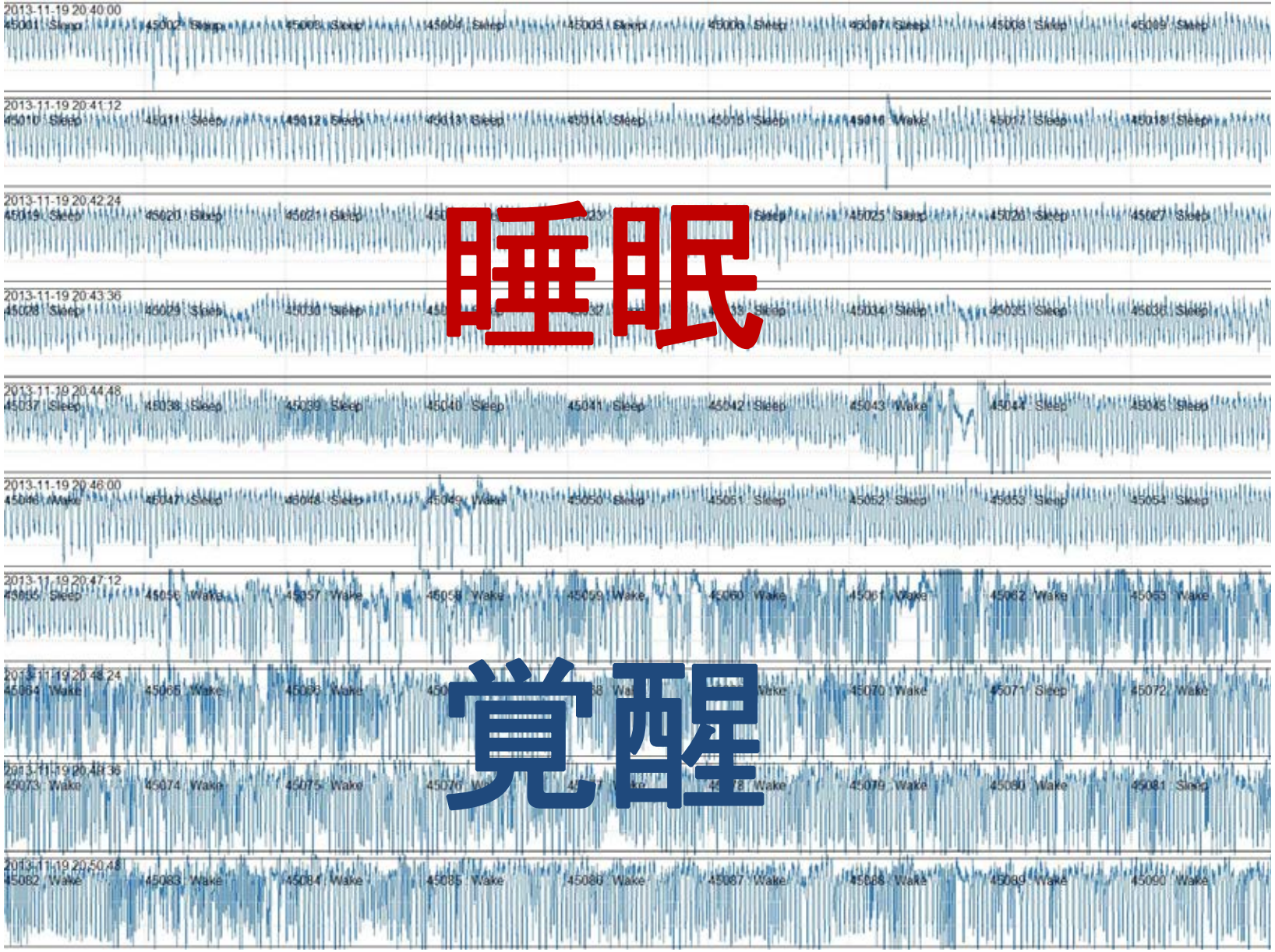
NREM睡眠・REM睡眠に重要な遺伝子の発見


脳波・筋電図による測定
ダメージ（侵襲的）
時間がかかる
難しい（高度なスキル）
非侵襲


ヒトによる睡眠ステージ判定
主観的
時間がかかる
退屈
自動化


200.000mV/Div
0.100sec/Div
344.500sec
0.000mV

マウスの睡眠覚醒状態を呼吸で判別可能である



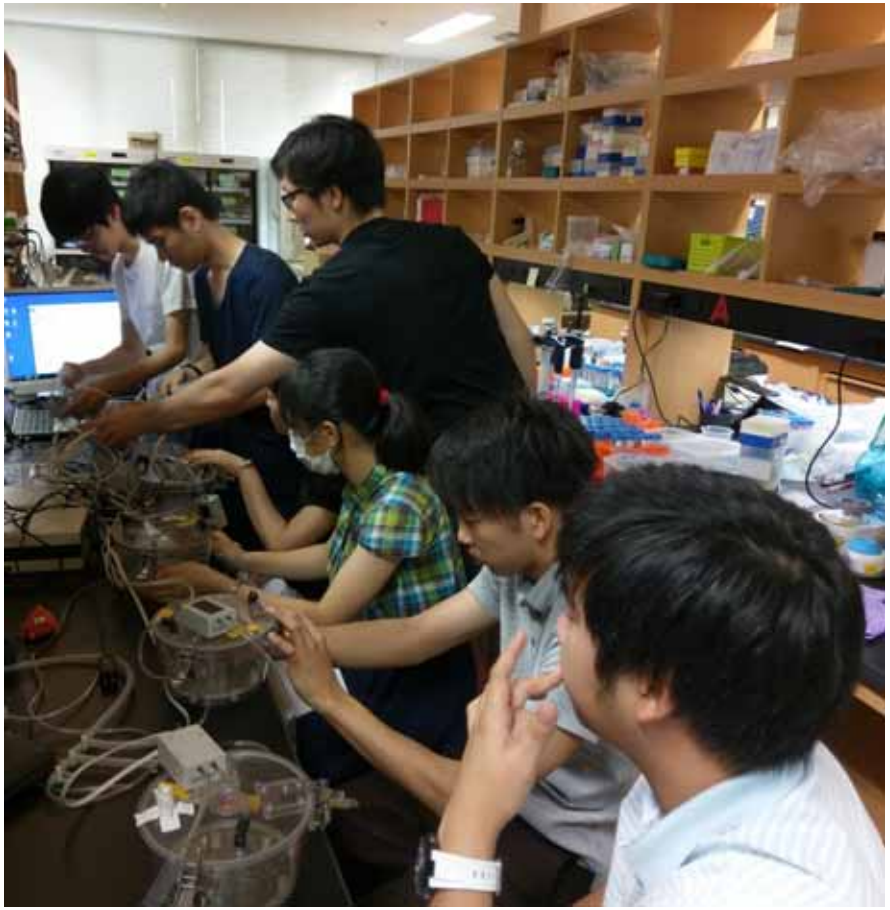
Animal Facility at RIKEN

Animal experiments at UTokyo

216 mice/week

医学教育

学部１年から睡眠測定を体感する



NREM睡眠・REM睡眠に重要な遺伝子の発見

ノンレム睡眠：カルシウムの重要性を提唱

Ca^{2+}：脳を興奮させる物質

↓

Ca^{2+}：脳を休ませる物質

CUBICによる脳の透明化

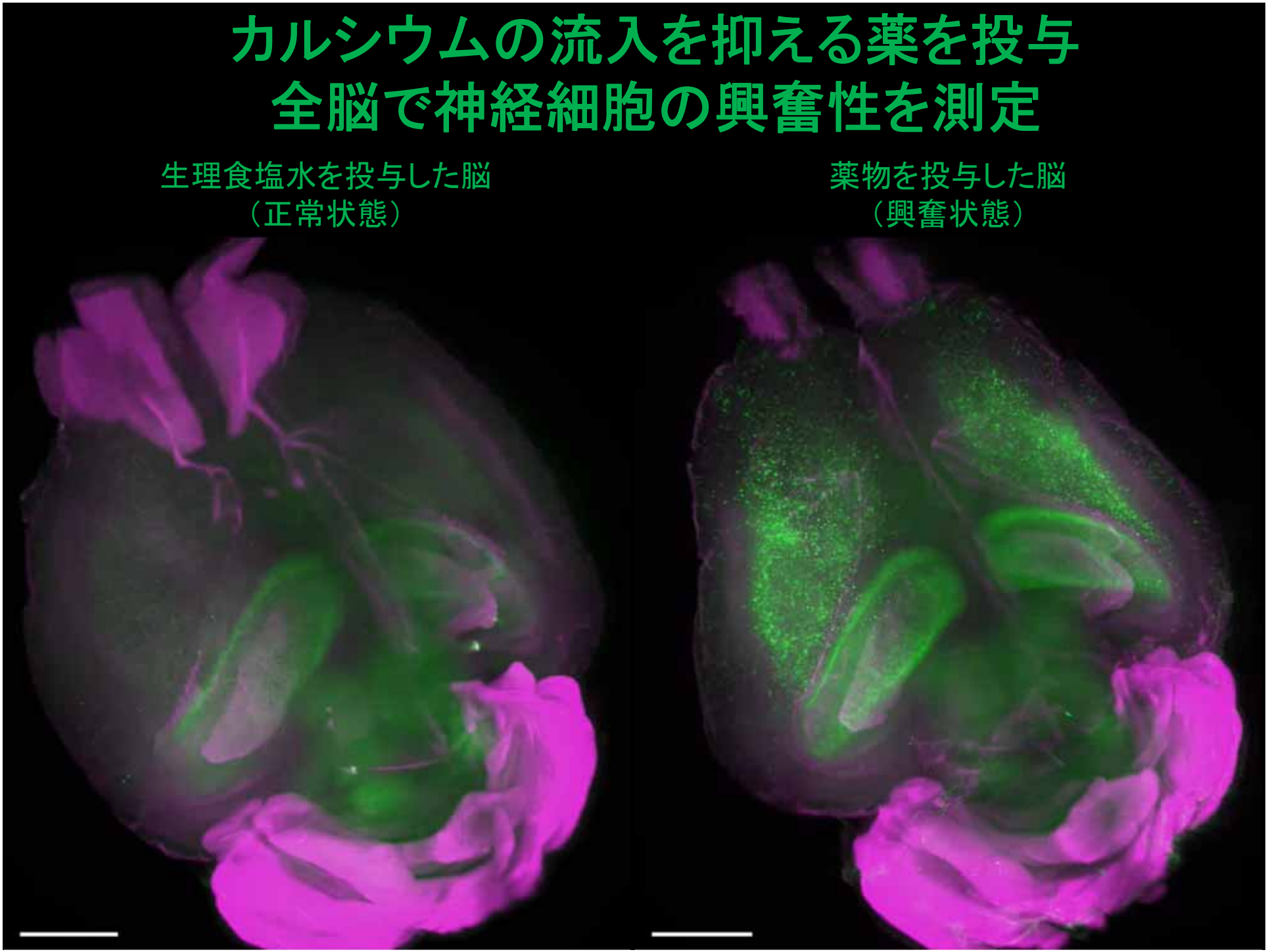
Whole-Brain Imaging with Single-Cell Resolution Using Chemical Cocktails and Computational Analysis

Susaki et al, Cell
(April 24th, 2014)

カルシウムの流入を抑える薬を投与
全脳で神経細胞の興奮性を測定

生理食塩水を投与した脳
（正常状態）

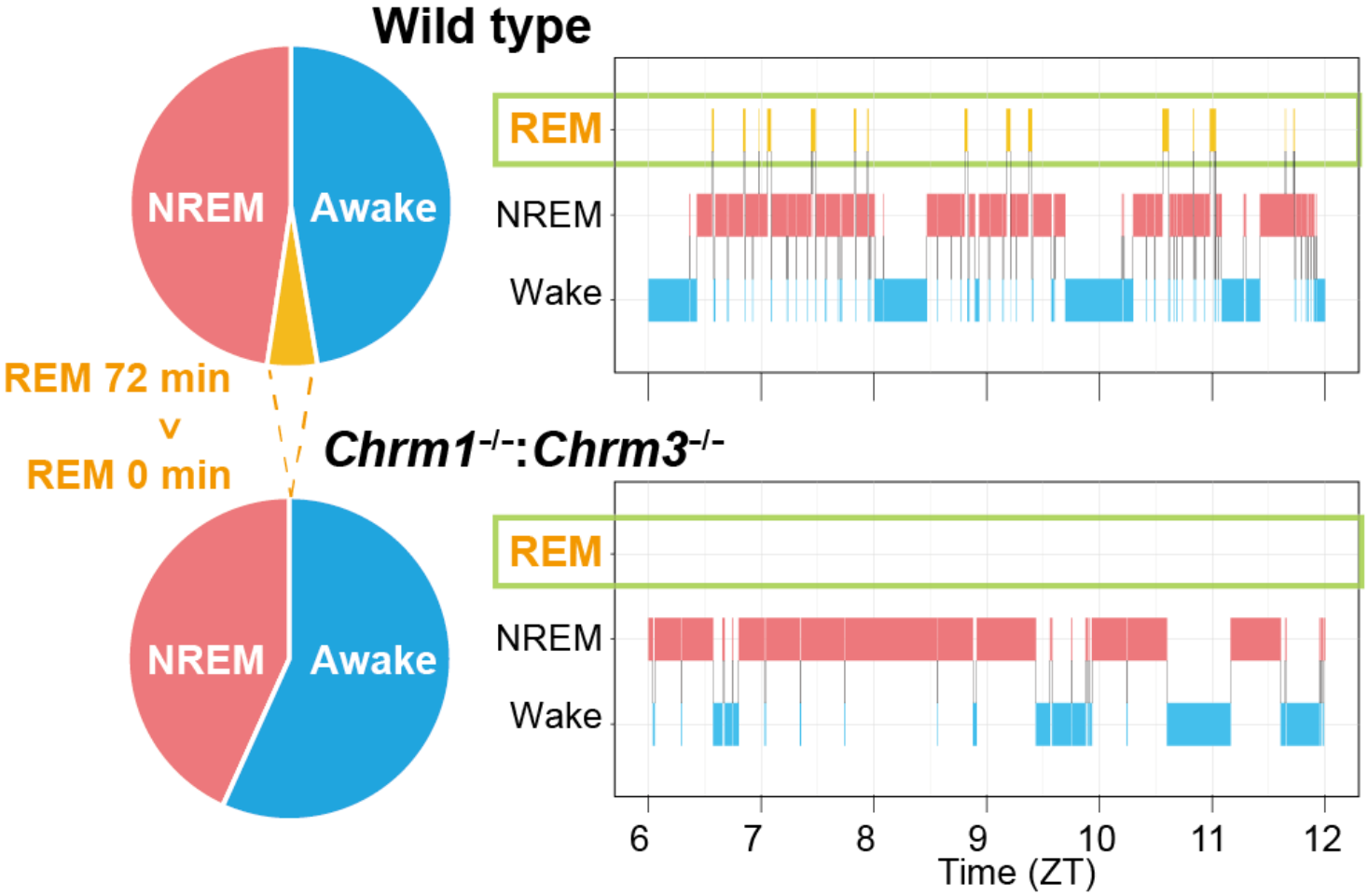
薬物を投与した脳
（興奮状態）



Rikuhiro Yamada

M1・M3の欠損マウスではレム睡眠がゼロに

Genki Kanda



レム睡眠遺伝子発見の国内外での反響

Newsweek — How To Sleep: Ability To Dream May Be In The Genes (Aug 31, 2018)

CBC — The Ability To Dream May Be Genetic (Aug 30, 2018, Canada)

GEN Genetic Engineering & Biotechnology News — Dream Genes Needed for REM Sleep (Aug 29, 2018)

EL ESPECTADOR — Los Genes Que Podrían Regular Cuánto Soñamos Fueron Descubiertos En Ratones (Aug 29, 2018, Spain)

LIVESCIENCE — Your Dreams May Come from These Two Genes (Aug 29, 2018)

SCIENTIFIC AMERICAN للعلم — اكتشاف الجينات المُنظمة للأحلام (Sep 2, 2018)

sina 新浪科技 — 日有所思夜有所梦？Cell子刊揭示:做梦的关键基因 (Sep 5, 2018, China)

la Repubblica.it — Ecco I Geni Che Regolano I Sogni: L'interruttore Del Sonno Rem (Aug 28, 2018, Italy)

THE NEW INDIAN EXPRESS — Gene Discovery May Help 'Turn Off' Nightmares (Aug 29, 2018, India)

BT — ‘Dreaming Genes’ Uncovered By Scientists (Aug 28, 2018, UK)

NAKED SCIENCE — Биологи обнаружили гены, необходимые для сновидений (Aug 29, 2018, Russia)

読売新聞（9月3日）

毎日新聞（8月29日）

朝日新聞（8月　日）

日本経済新聞（8月29日）

日刊工業新聞（8月29日）

化学工業日報（8月29日）

日経産業新聞（8月29日）

マイナビニュース（9月3日）

(Altmetric on Sep 13, 2018)

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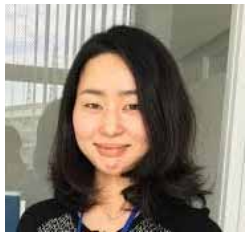

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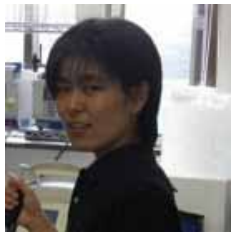

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