

环境污染物的生物效应评价

冯起 DAS LST 技术经理

2018年8月14日



HUMAN HEALTH • ENVIRONMENTAL HEALTH

© 2014 PerkinElmer

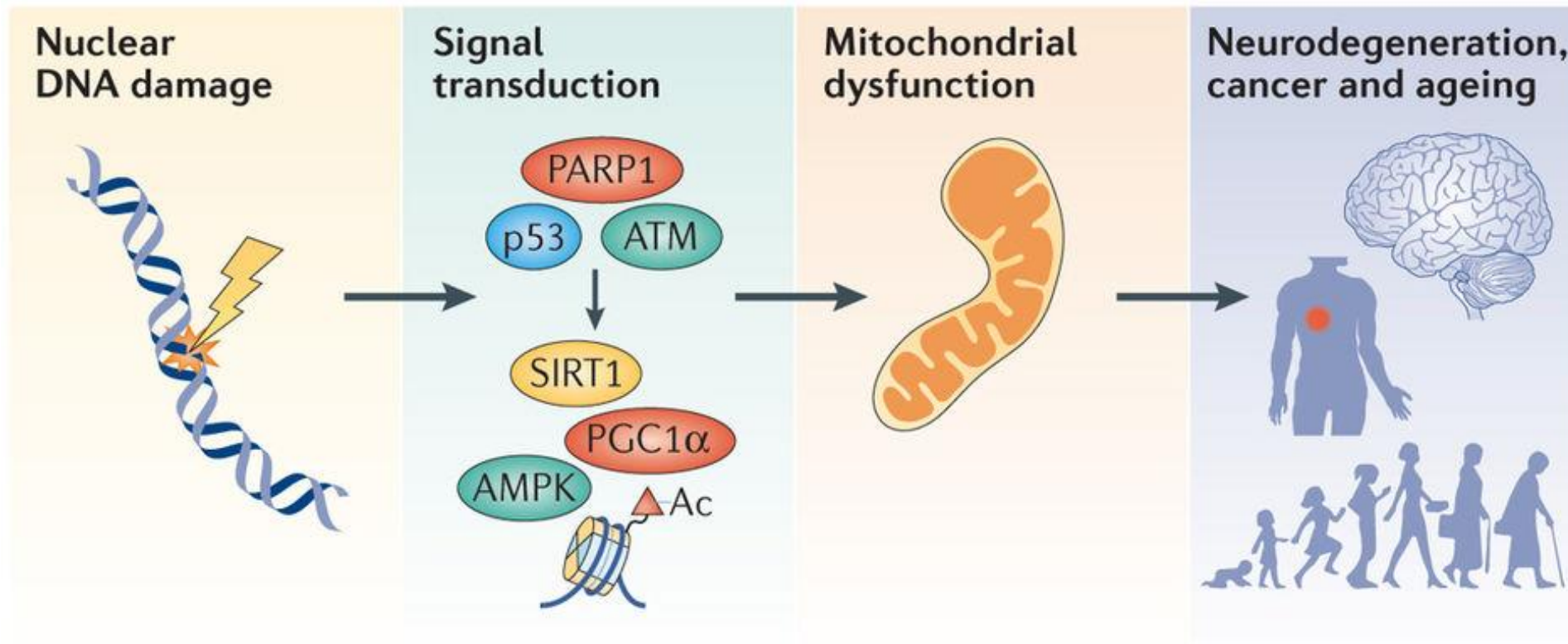
环境污染影响人类健康

无机污染物

有机污染物

颗粒污染物

放射性污染



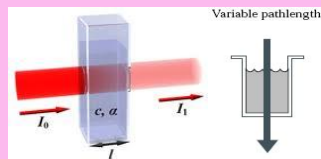
PerkinElmer生命科学研究方案

应用方向



环境污染生物评价之多功能酶标技术

光吸收
(Abs)



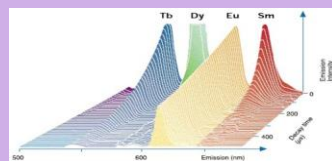
荧光检测 (FI)



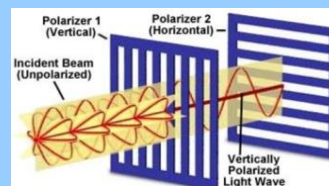
化学发光
(Lumi)



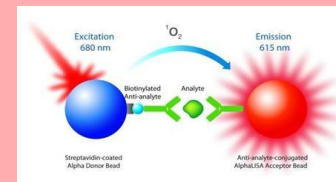
时间分辨荧光
(TRF)



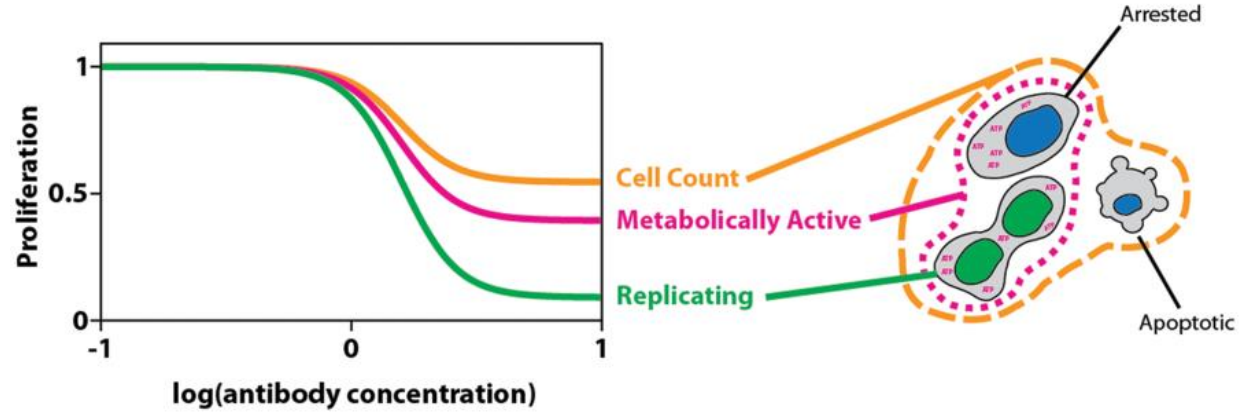
荧光偏振
(FP)



Alpha技术



细胞增殖 细胞毒性 细胞死亡

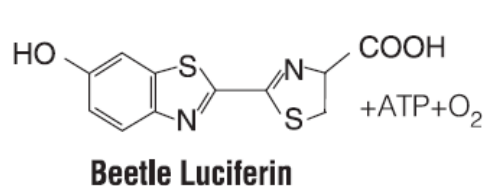
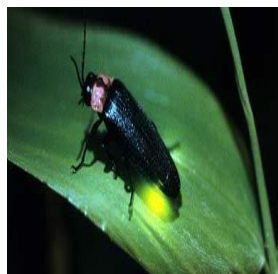


| 检测技术 | 主要实验方法 | 灵敏度 | 背景噪声 | 特异性 | 实验周期 |
|---------|---|-----|------|-----|--------|
| 光吸收 | MTT类检测 (490 nm) CCK8 (WST-8) 检测 (450 nm) LDH检测 (500 nm) SRB检测 (540 nm) | 低 | 中 | 中等 | 1-2小时 |
| 荧光强度 | 阿尔玛蓝 (Alamar Blue) (530/590 nm) Calcein-AM (活细胞荧光染料) | 中等 | 高 | 中等 | >2小时 |
| 化学发光法 | ATP检测法ATPLite 细胞色素P450酶活检测 | 高 | 低 | 高 | < 20分钟 |
| 时间分辨荧光 | DNA片段化检测 (DELFI DNA fragmentation) DNA复制检测 (DELFI BrdU Proliferation) 细胞膜通透性检测 (DELFI BATDA Cytotoxicity) | 高 | 低 | 高 | 1-2小时 |
| Alpha检测 | Caspase 等特异性凋亡Marker | 高 | 低 | 高 | 1-2 小时 |
| 细胞荧光成像 | 通过明场或者细胞特异性染料染色 如Annexin V等特异性凋亡Marker | 高 | 中 | 高 | >2小时 |

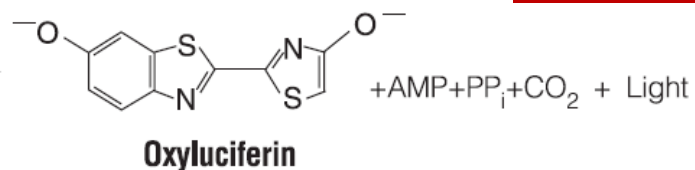


多功能酶标仪 & 高内涵成像系统

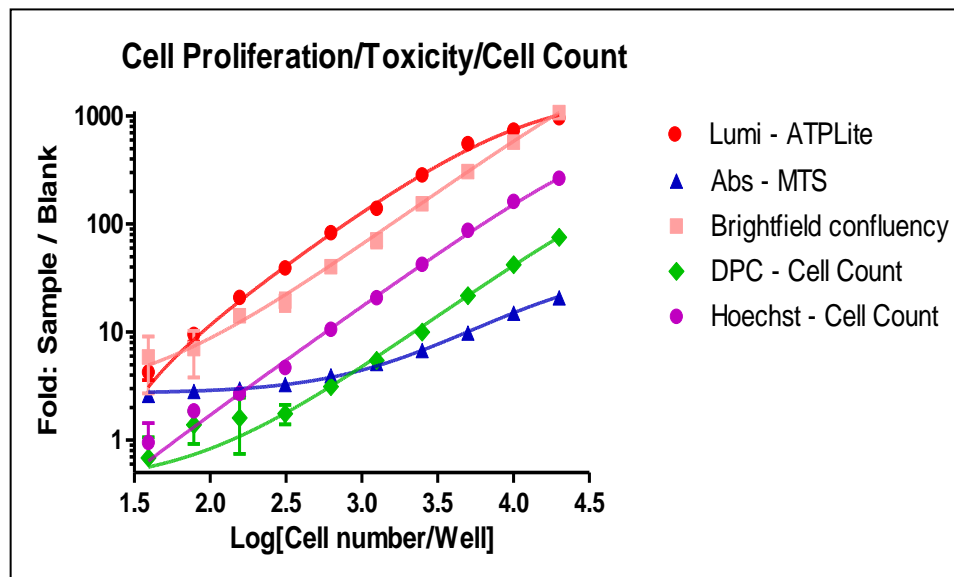
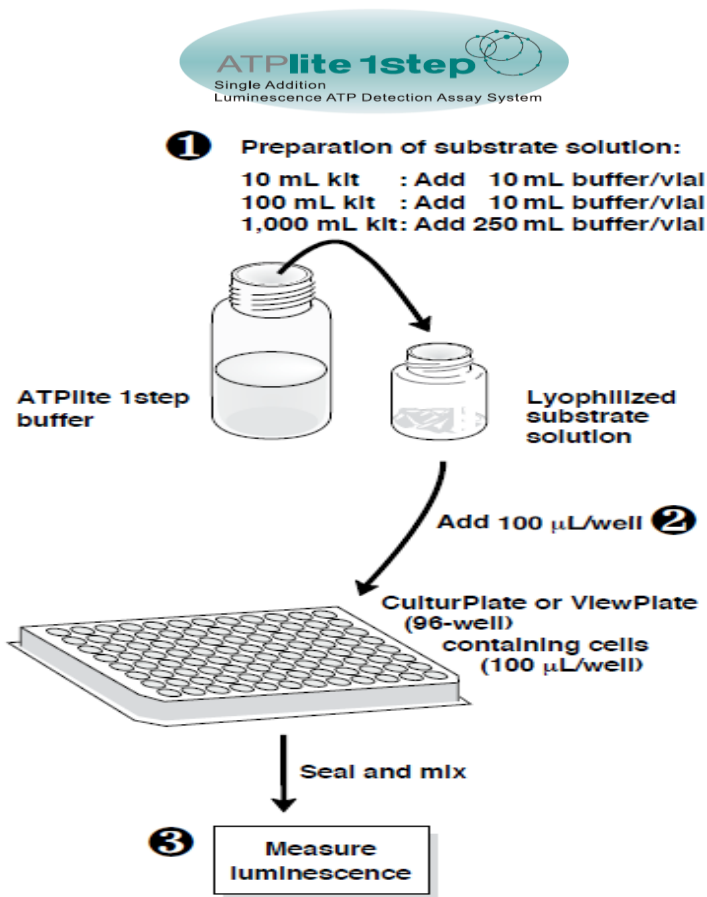
ATPlite 1step™ 细胞活性检测



Firefly
Luciferase
Mg²⁺

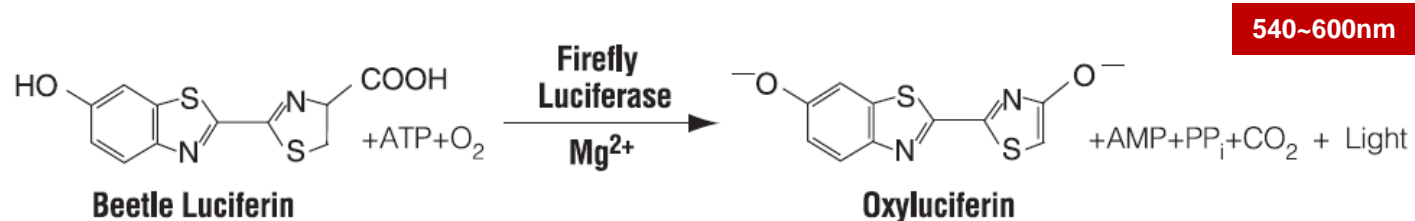
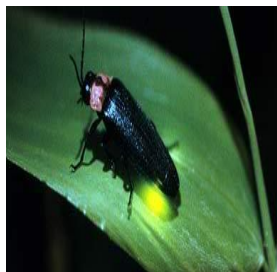


540~600nm



- ❖ 检测灵敏更高 (可检测到个位数细胞)
- ❖ 更高的动态检测范围, 高信噪比
- ❖ 抗干扰能力强, 数据稳定

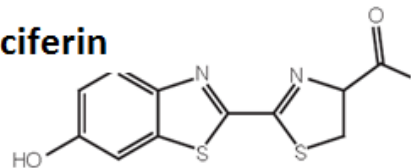
化学发光进行P450药代研究



**P450-Glo™ Substrate
(proluciferin)**

CYP Enzyme

Luciferin

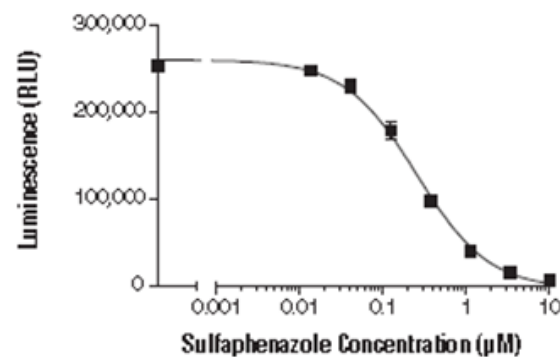


Luciferin + O₂ + ATP

Luciferase

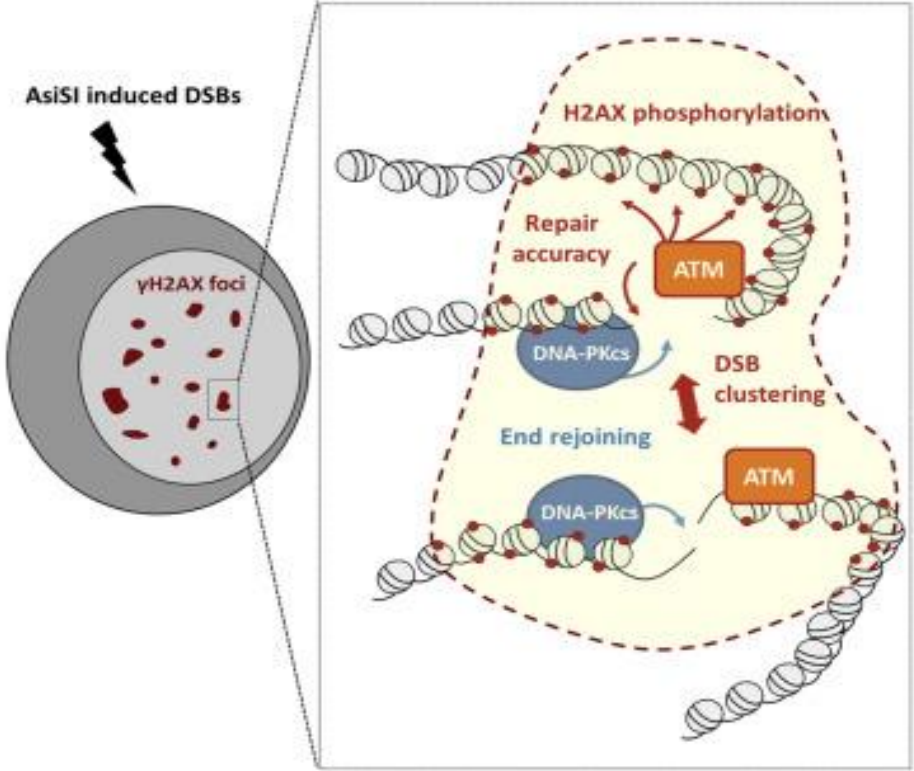
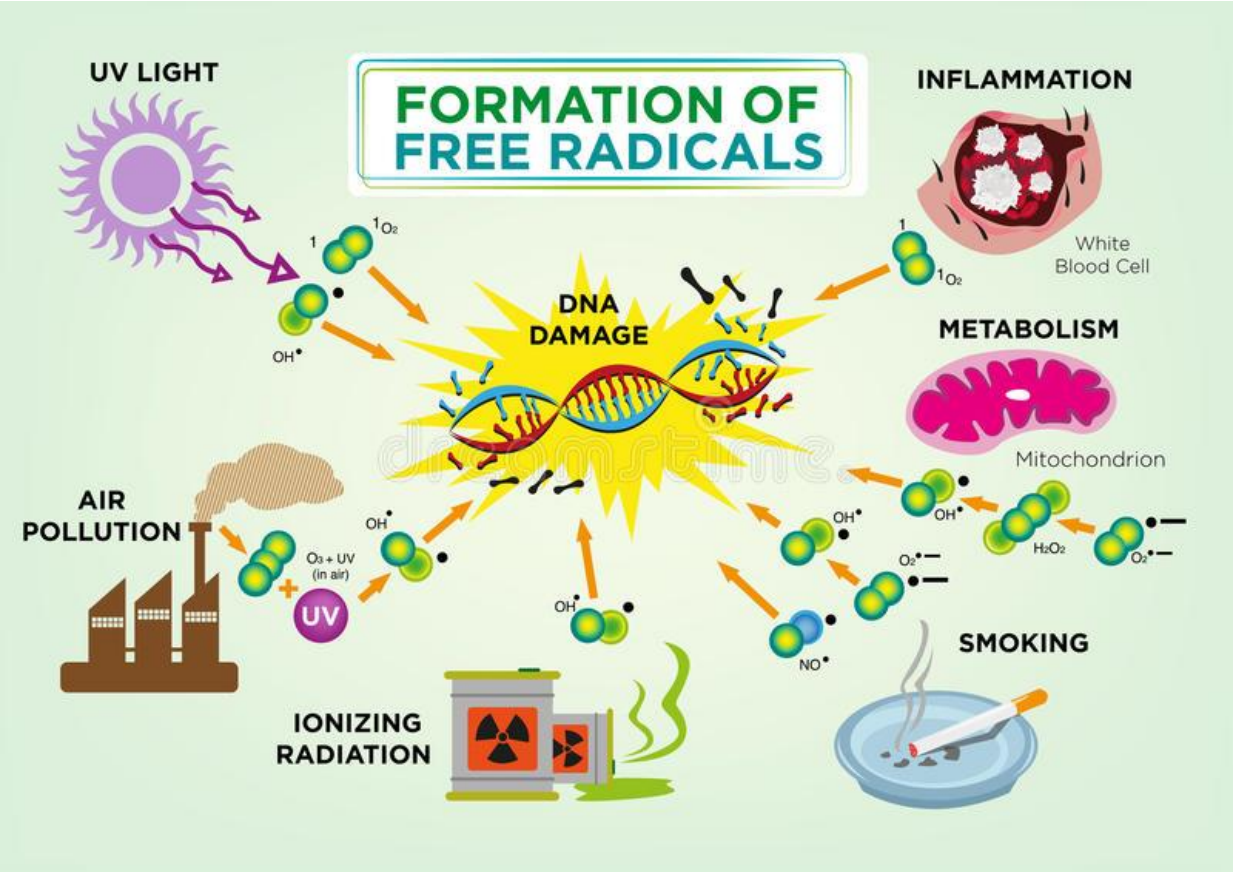
Mg²⁺

Glo



细胞色素P450，隶属亚铁血红素蛋白家族，参与内源性或药物分子的代谢，用于药代研究。

组蛋白H2AX磷酸化抗体特异检测双链DNA断裂





Available online at www.sciencedirect.com

ScienceDirect

www.journals.elsevier.com/journal-of-environmental-sciences



通过发光细菌的方法构建重金属离子的生物传感器

Application of internal standard method in recombinant luminescent bacteria test

Yong-zhi Wang¹, Dan Li², Miao He^{1,*}

1. Environmental Simulation and Pollution Control (ESPC) State Key Joint Laboratory, School of Environment, Tsinghua University, Beijing 100084, China. E-mail: wyz06@mails.tsinghua.edu.cn
2. Fudan University, Department of Environmental Science & Engineering, Shanghai 200433, China

发光细菌质粒的构建

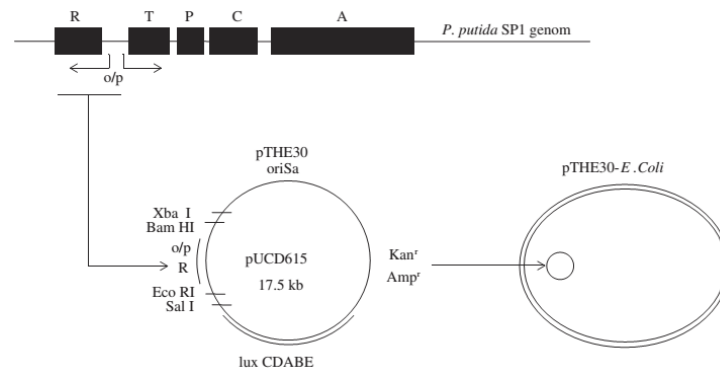


Fig. 1 – Construction of pTHE30-E. coli. Gene fragment of *mer* gene. R: mercury regulatory protein gene and o/p: the promoter/operator sequence from *P. putida* SP1 were fused upstream of promoter-less *lux* gene in pUCD615, then transformed to *E. coli* DH5 α .

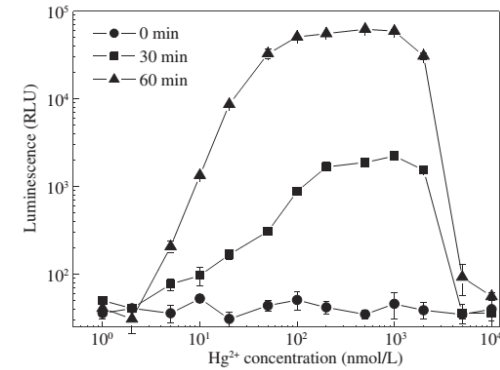


Fig. 2 – Luminescence (relative luminescence unit, RLU) of pTHE30-E. coli at different time after addition of Hg²⁺.

对汞离子的时间和剂量依赖效应

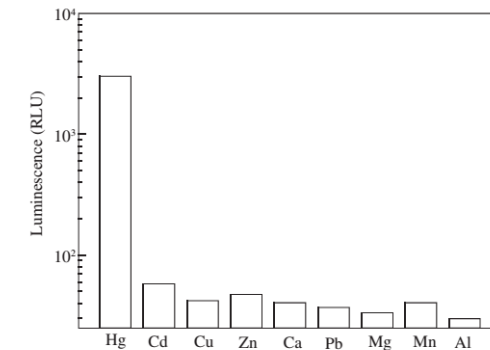
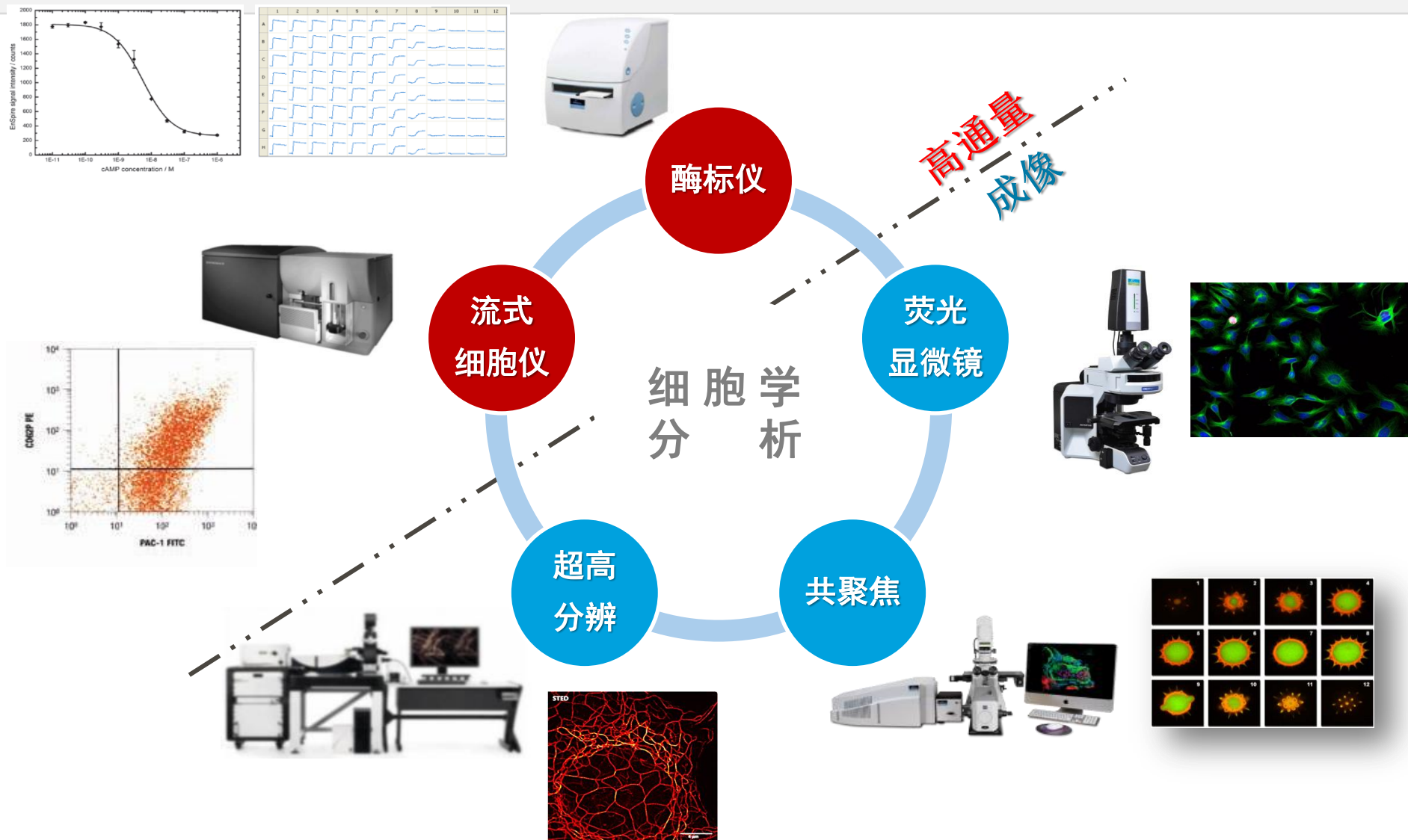


Fig. 3 – Specificity of pTHE30-E. coli in the presence of different heavy metals. Data shown were the maximum luminescence of heavy metal in a range of 10⁻⁵-1 mmol/L.

和其他重金属离子之间没有交叉反应

环境污染生物评价之高内涵技术

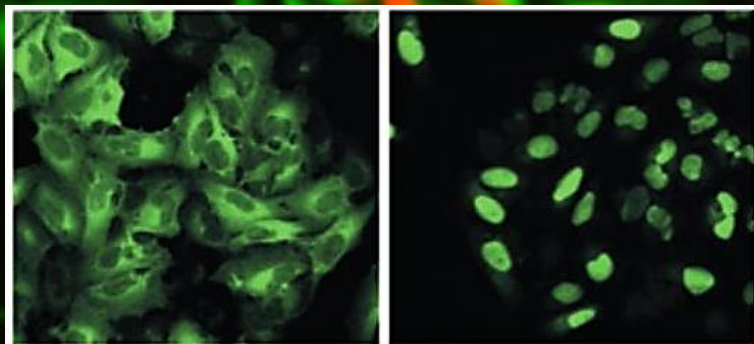


单细胞形态学 + 自动化高通量

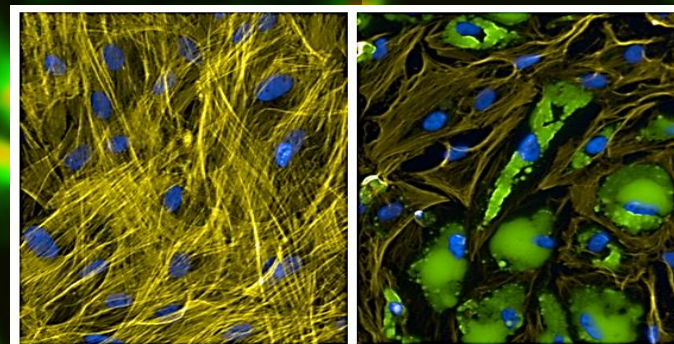


Operetta® CLS

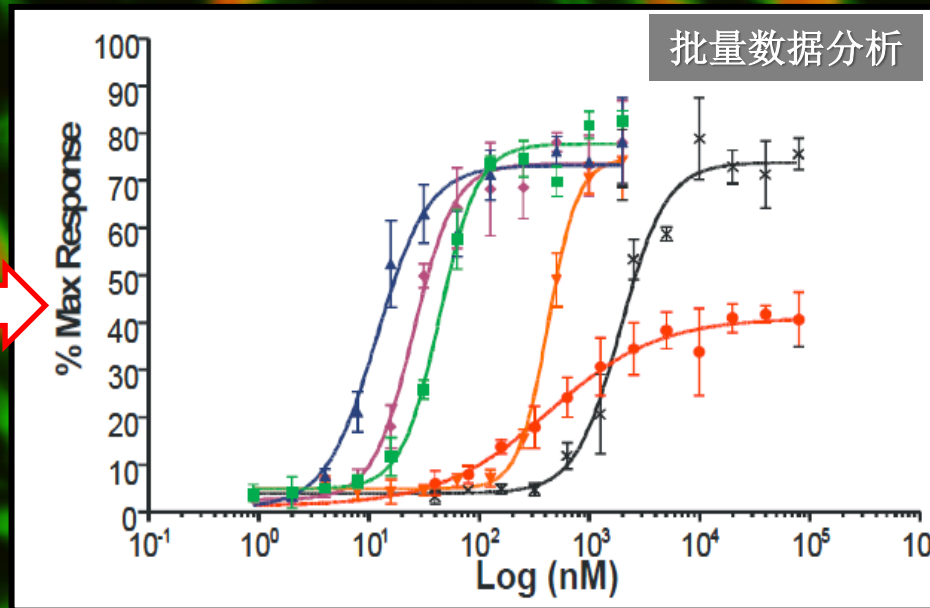
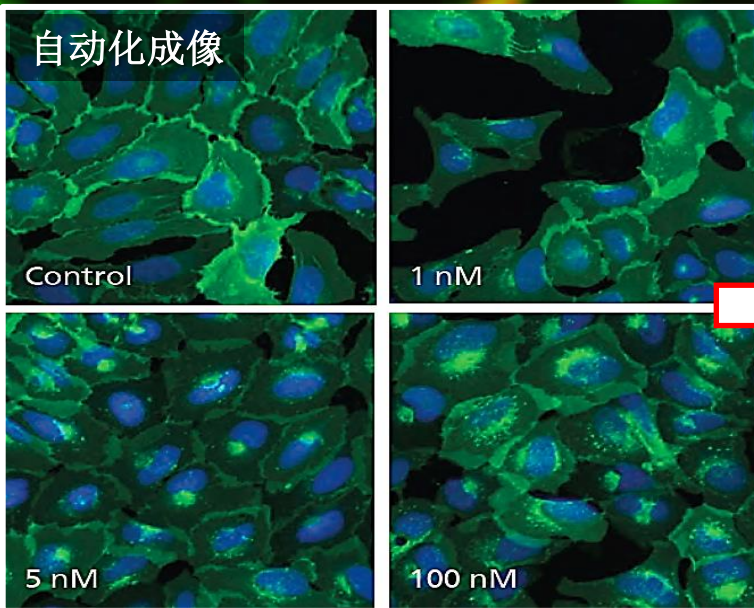
信号分布差异



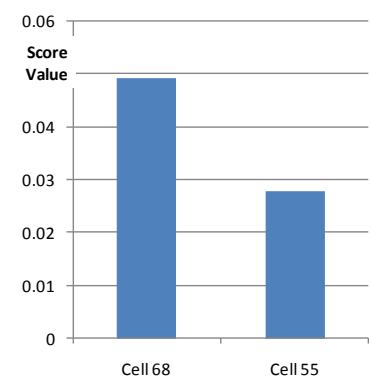
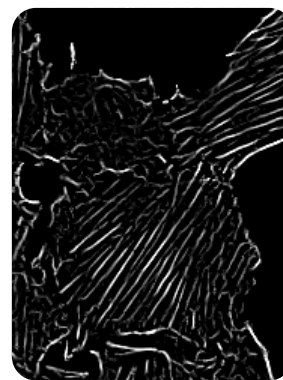
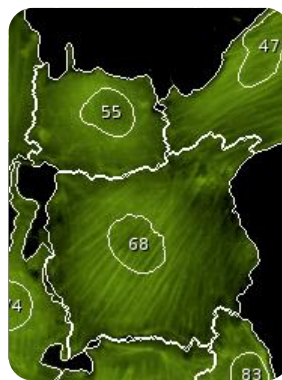
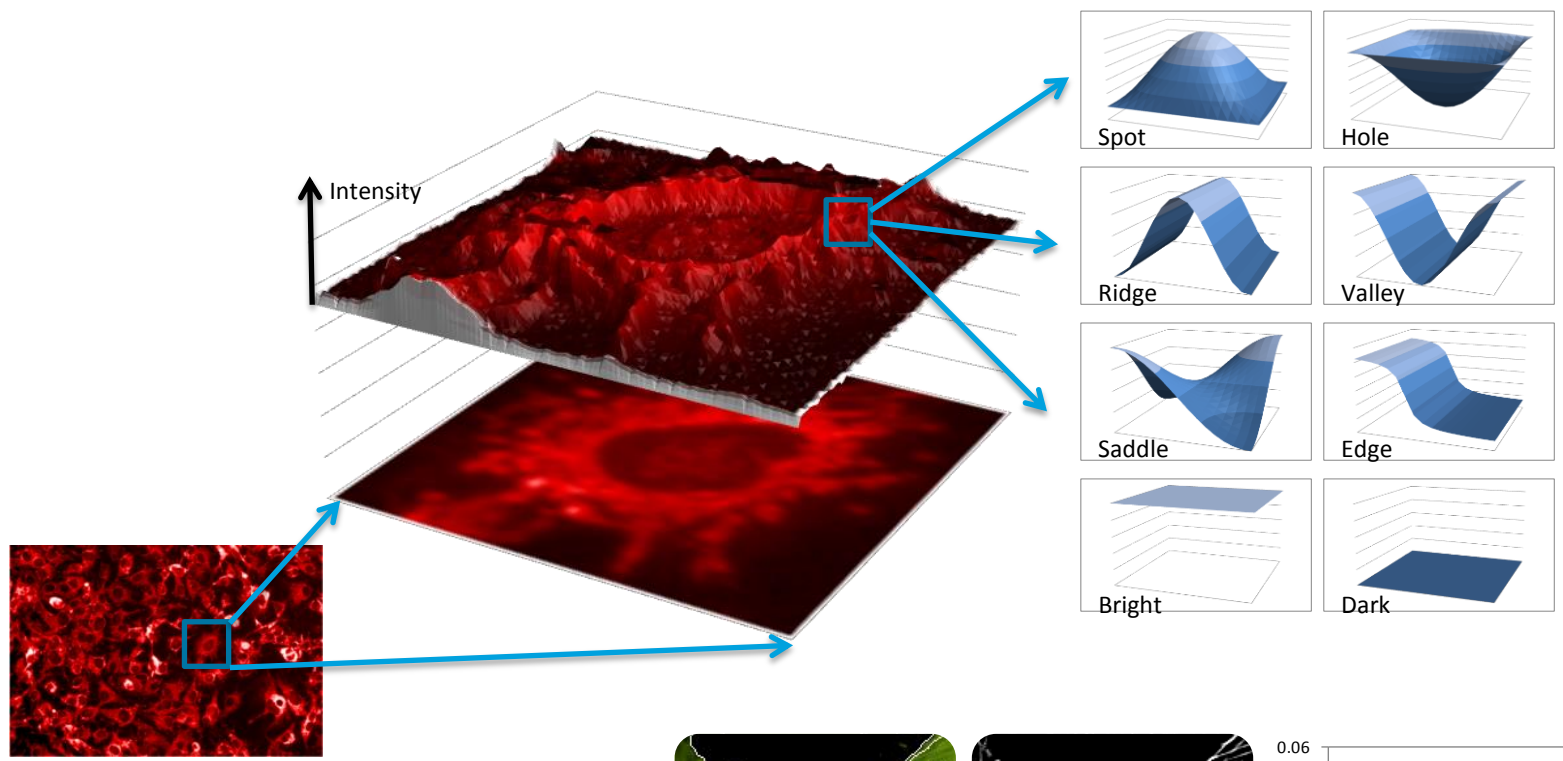
纹理结构特征差异



自动化成像

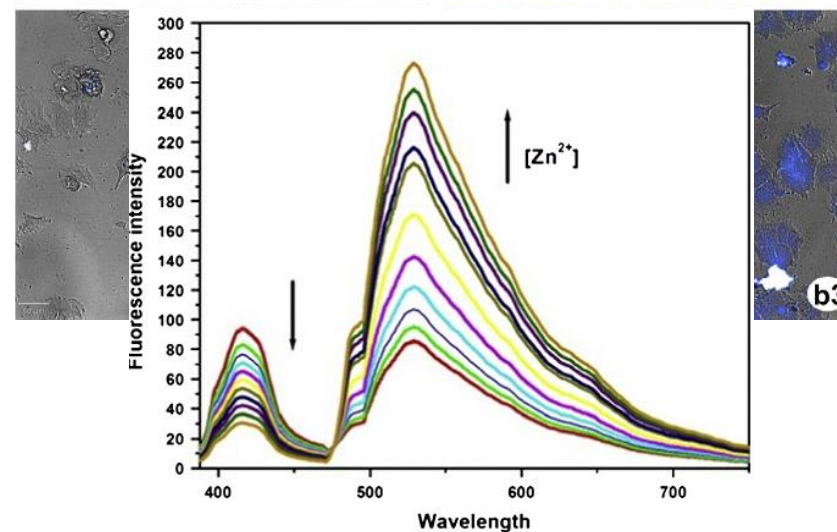
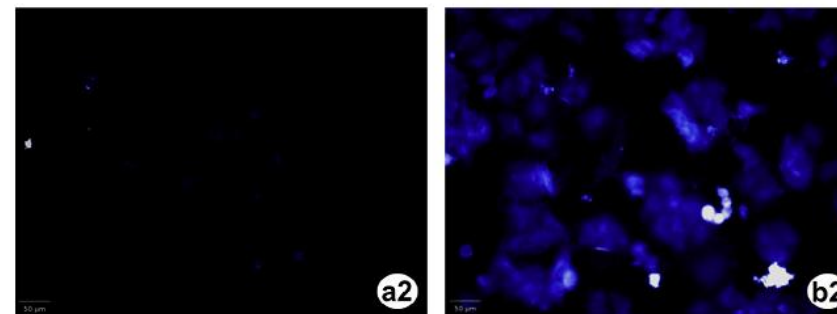
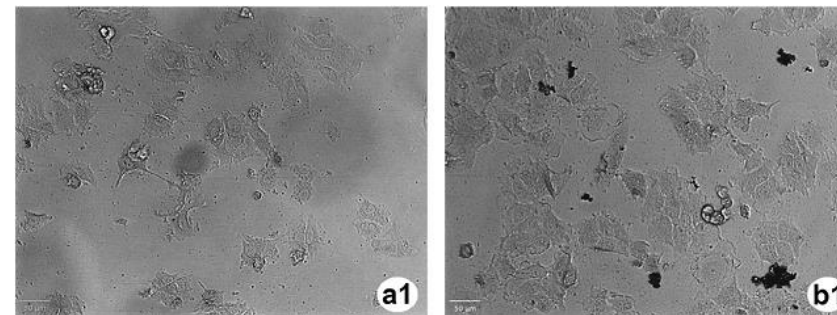
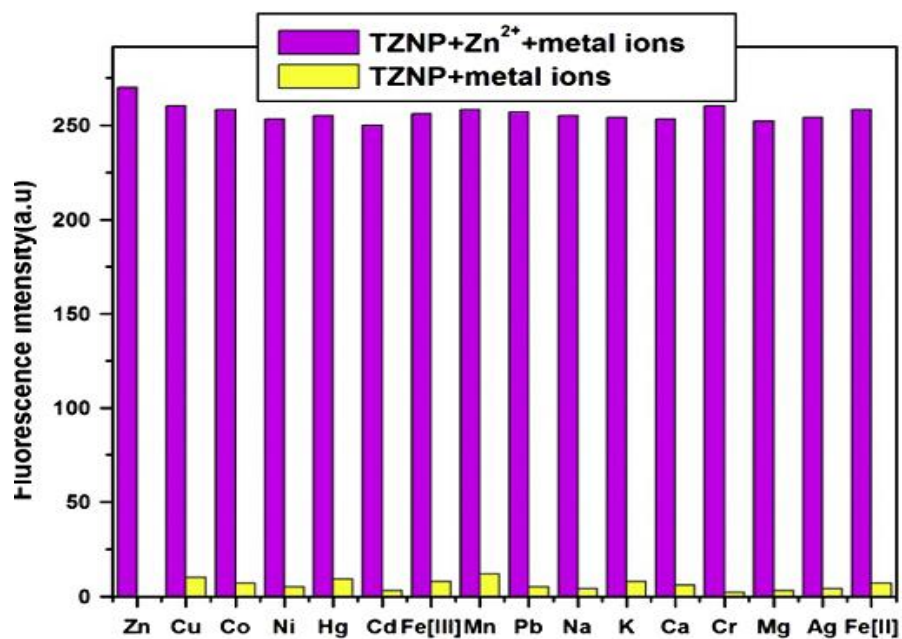
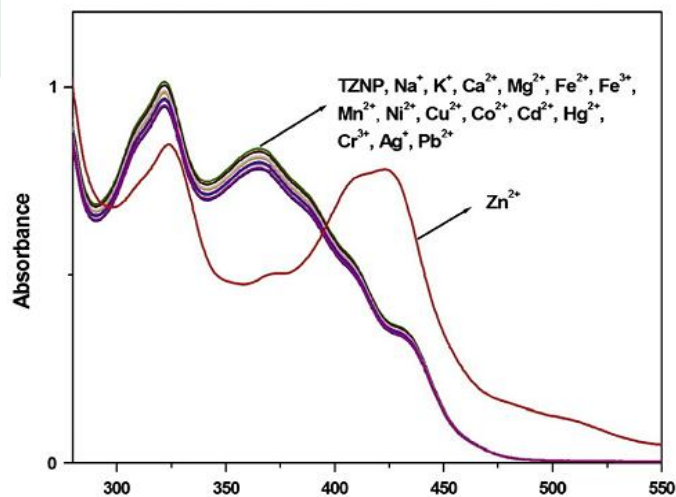


纹理分析：分析的不止是信号强度



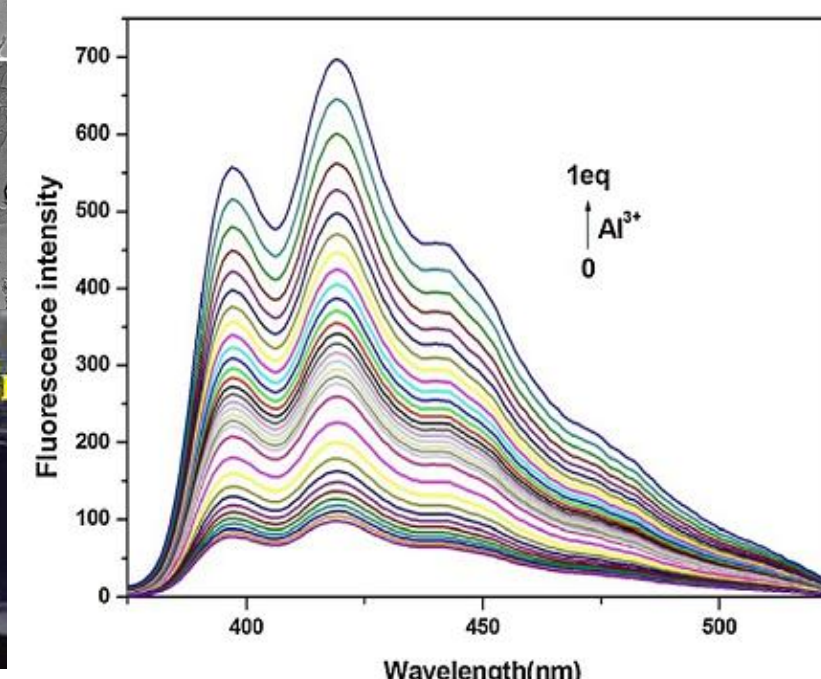
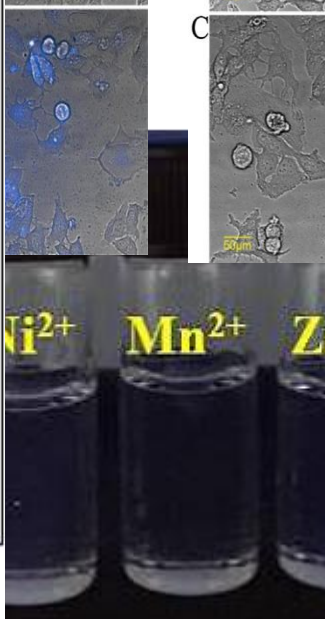
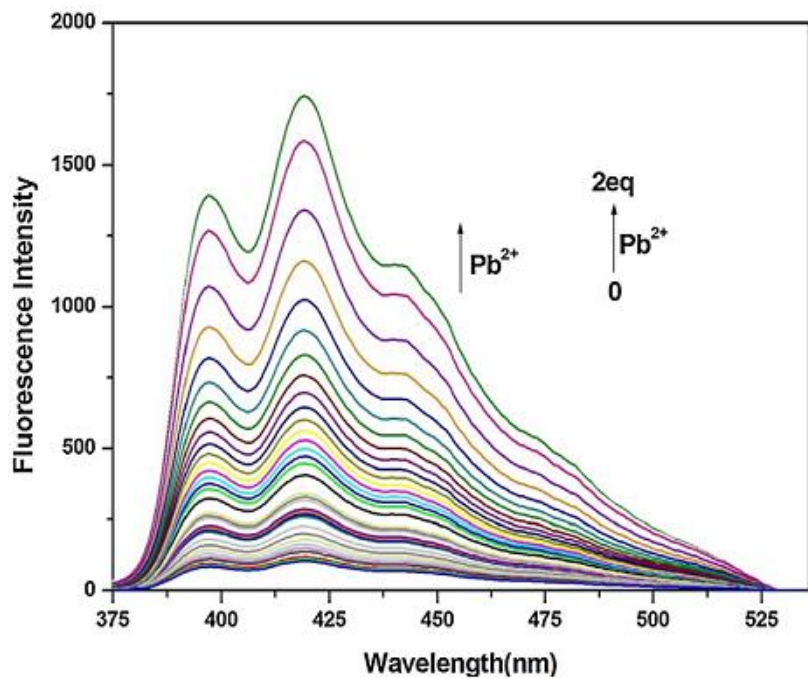
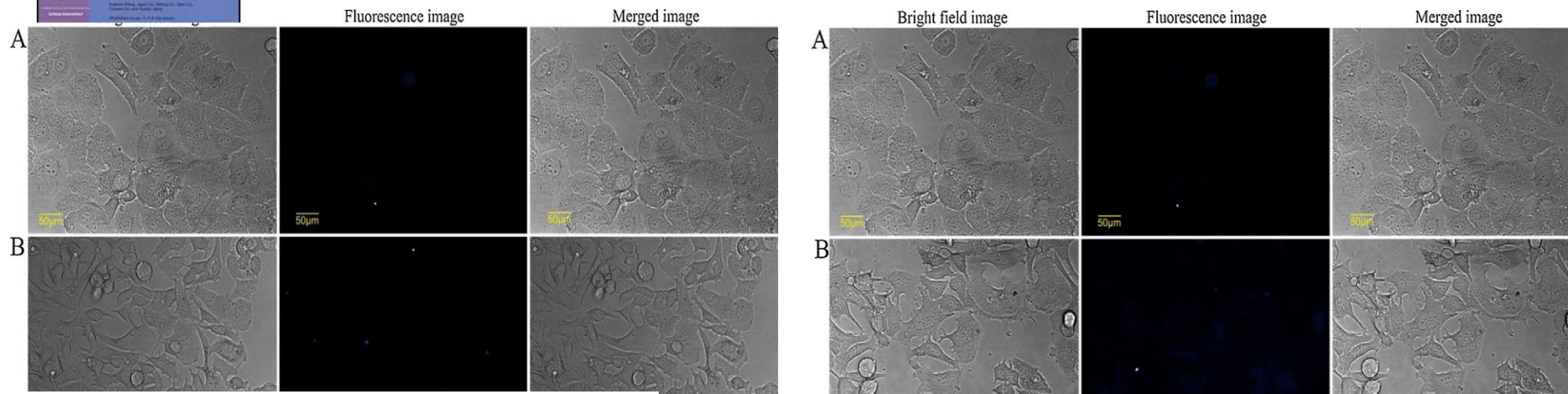
Source: Operetta™ Application Guide

Triazole based ratiometric fluorescent probe for Zn²⁺ and its application in bioimaging

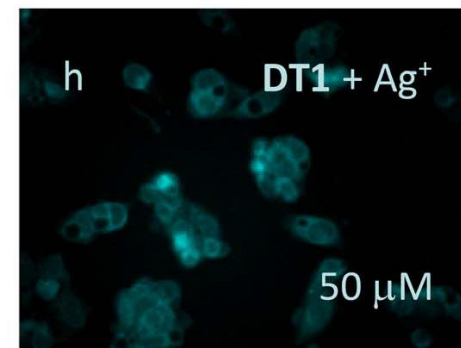
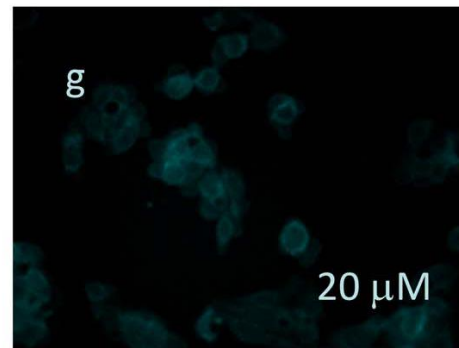
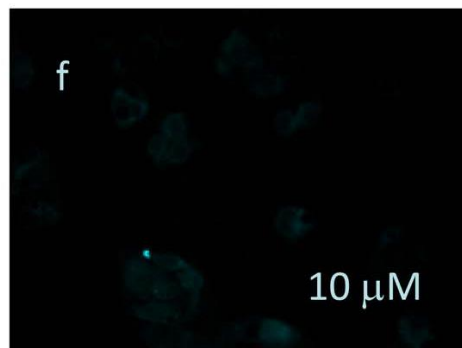
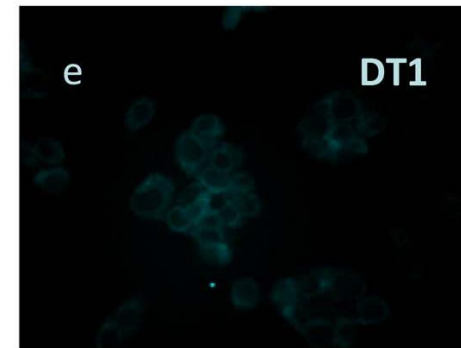
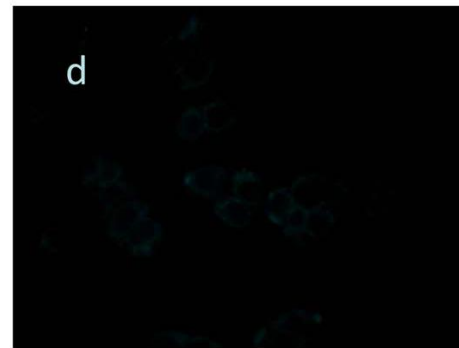
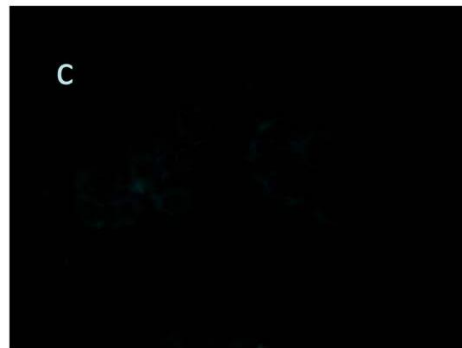
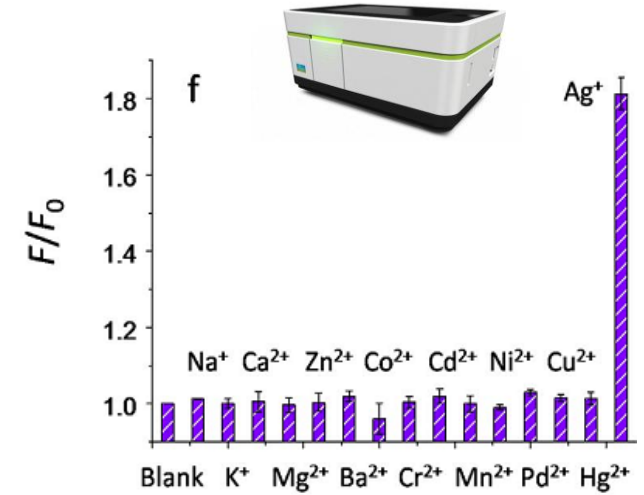
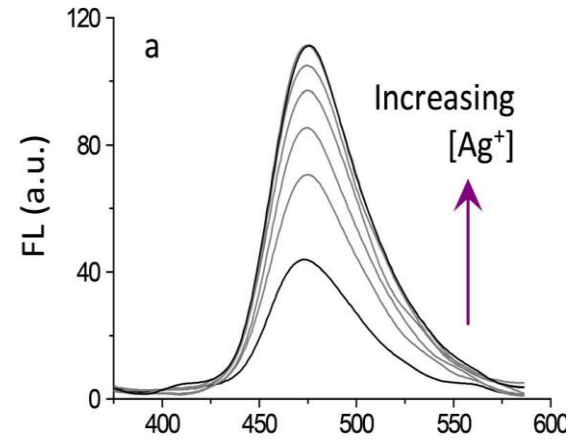
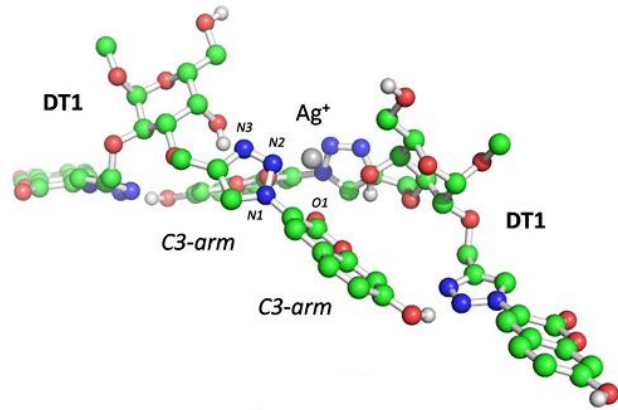




Aminoquinoline based highly sensitive fluorescent sensor for lead(II) and aluminum(III) and its application in live cell imaging



Substitution Pattern Reverses the Fluorescence Response of Coumarin Glycoligands upon Coordination with Silver(I)





Toxicology in the 21st Century

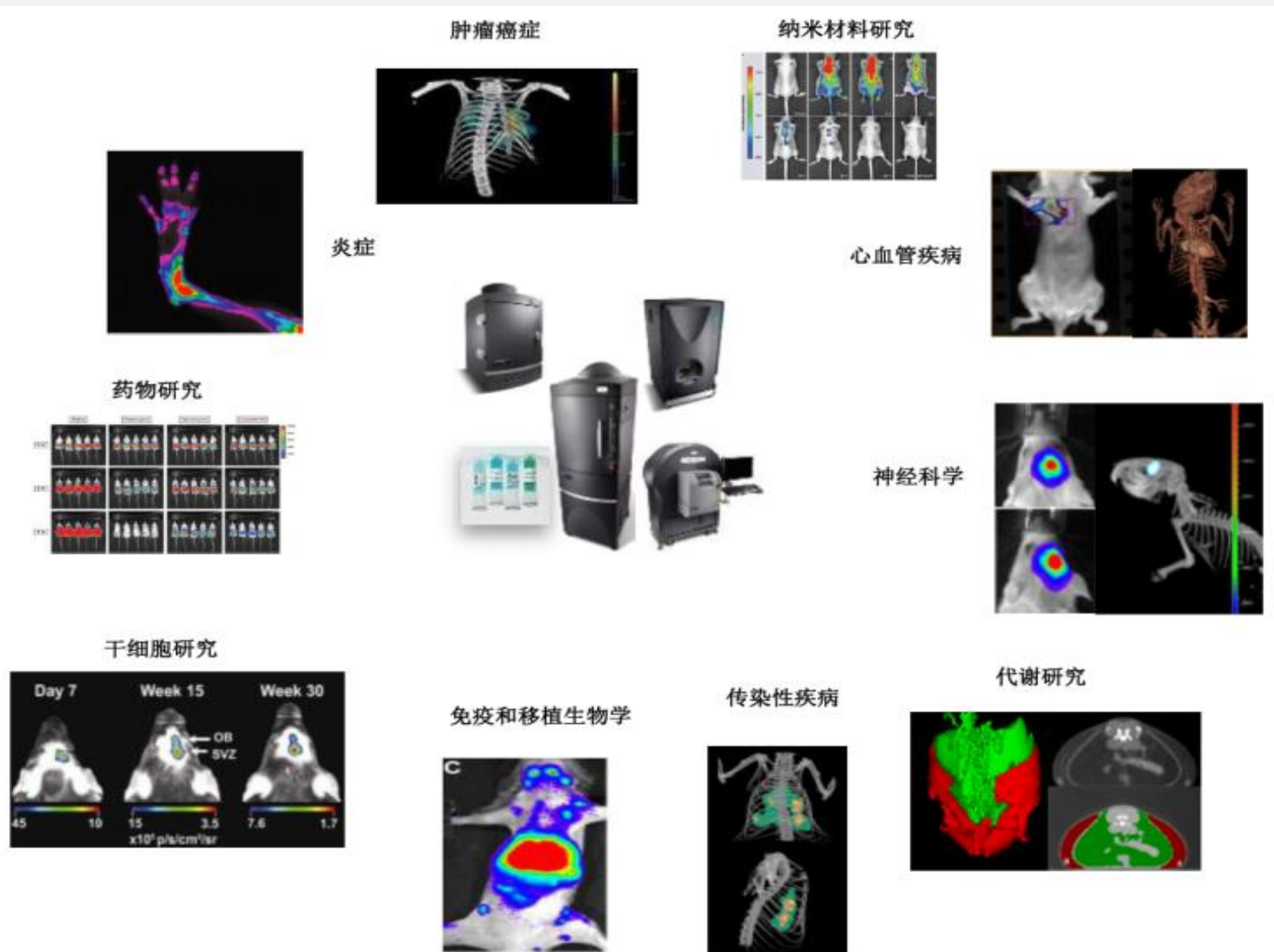
UNITED STATES FEDERAL GOVERNMENT COLLABORATION TOXICOLOGY IN THE 21ST CENTURY (TOX21)

TESTING THOUSANDS OF ENVIRONMENTAL CHEMICALS USING NON-ANIMAL METHODS

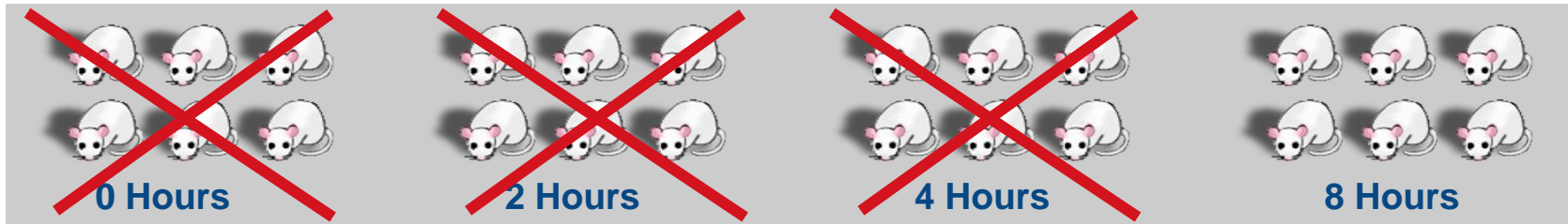
- Toxicology in the 21st Century (Tox21) is a US federal research collaboration that is developing alternative, non-animal methods to **quickly and efficiently test thousands of chemicals for potential health effects**.
- Since its formation in 2008, Tox21 has screened approximately **10,000** chemicals in more than 70 rapid tests called “qualitative high-throughput screening assays”. This includes chemicals used in industrial processes and consumer products as well as **food additives, approved and investigational drugs, and chemical mixtures**.
- Tox21 has published over **200 scientific peer-reviewed articles** in approximately 55 journals. Articles were most frequently published in Toxicological Sciences, Environmental Health Perspectives, Chemical Research in Toxicology, and Environmental Science and Technology.



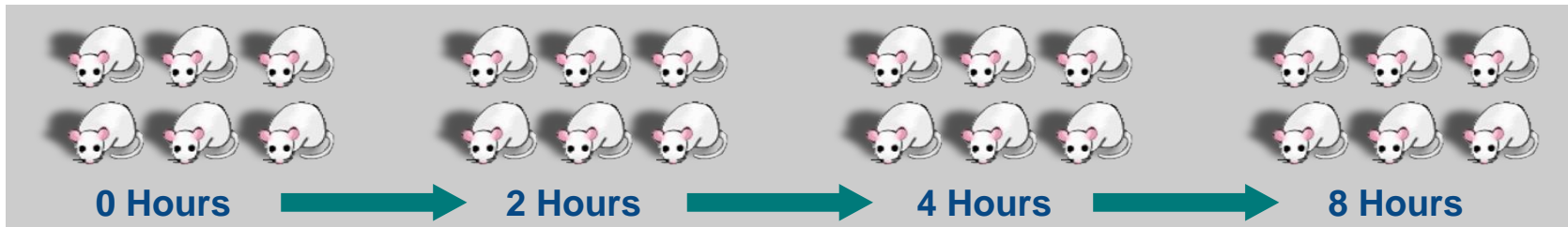
环境污染生物学评价之小动物活体成像技术



Current Methodology = 24 animals over four treatment points



In Vivo Imaging = the same 6 animals over four treatment points

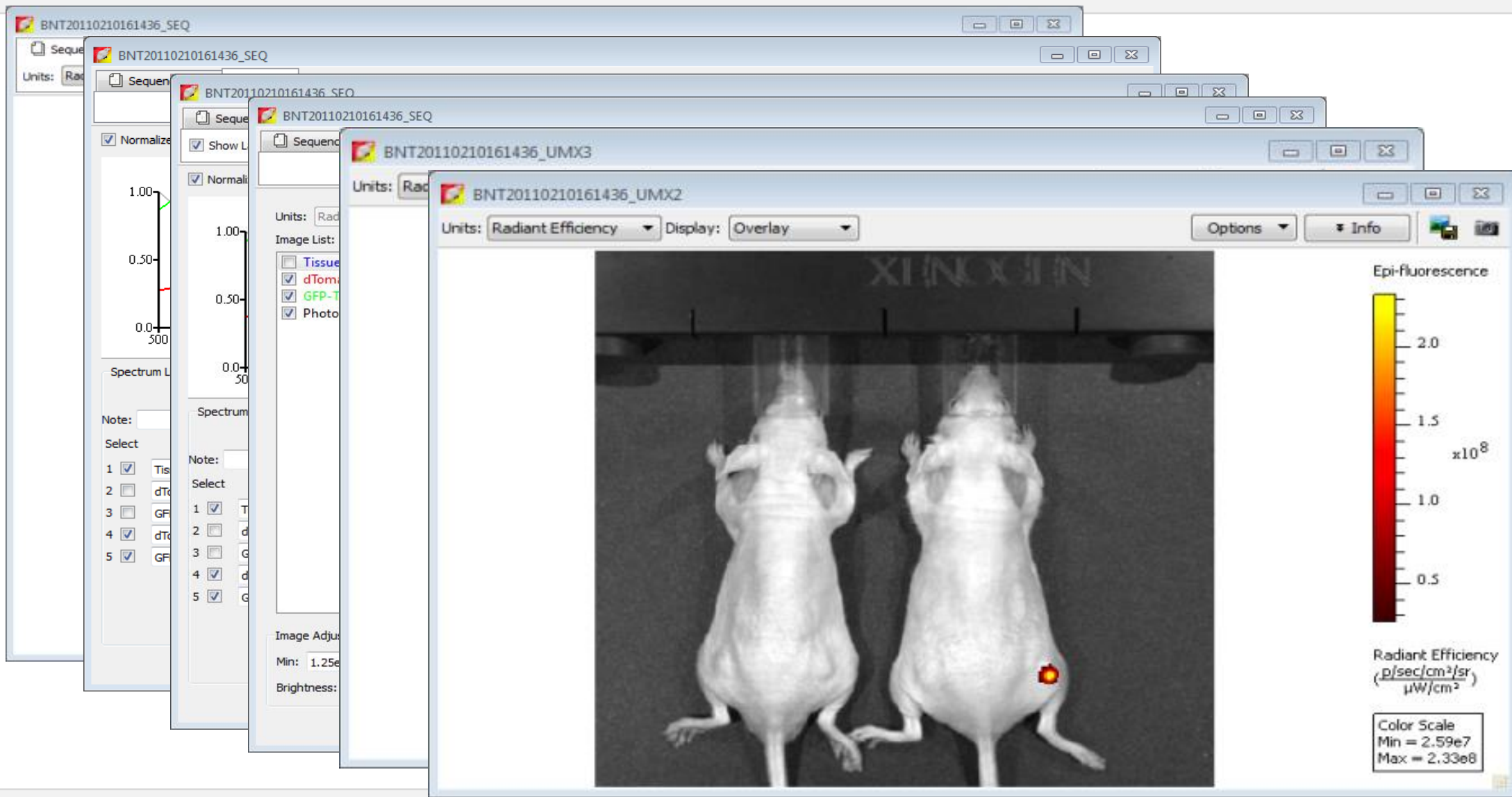


Example: 4 groups, 5 mice each group, 8 time points

Traditional methodology: **160 mice**

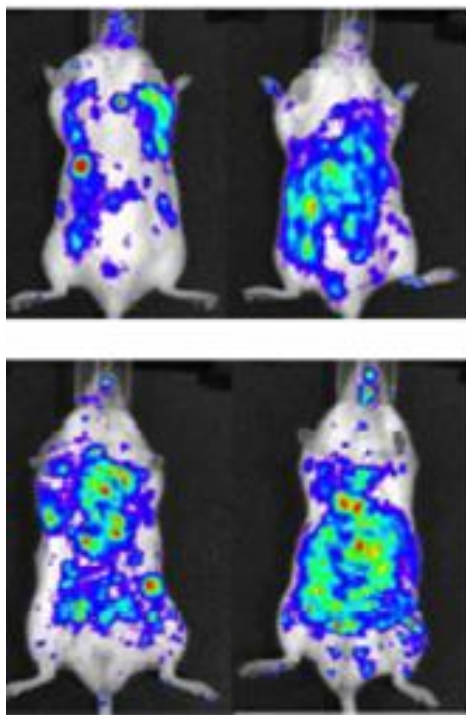
In vivo imaging: **20 mice**

多光谱分离技术能有效地去除背景噪音

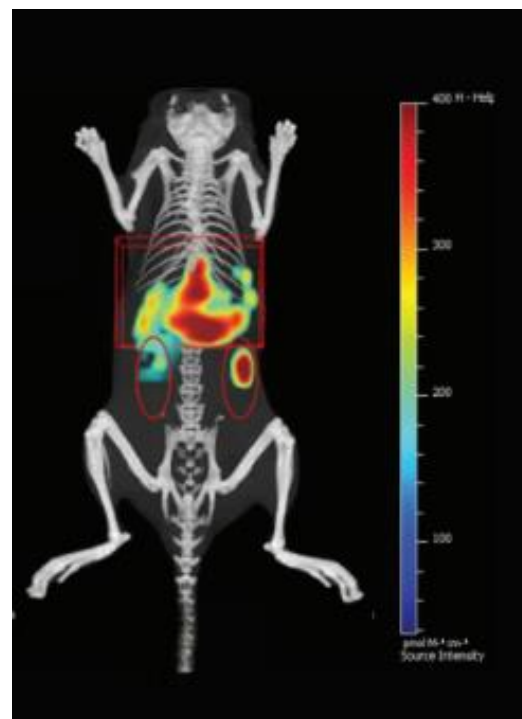


小动物模型的各种毒性评价

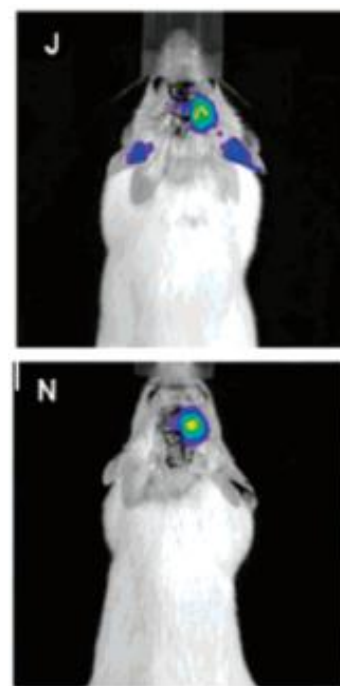
全身毒性



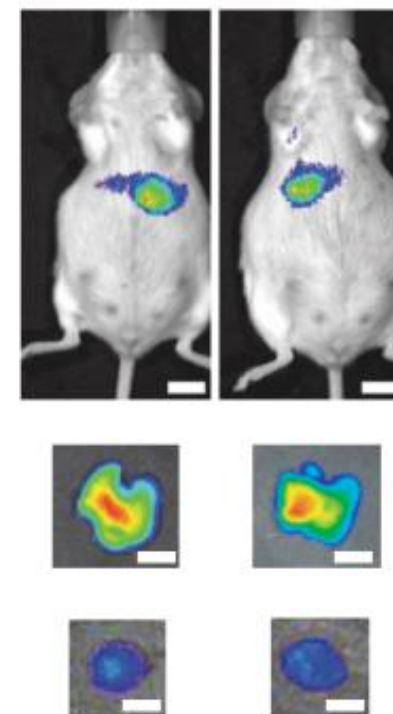
肝脏毒性



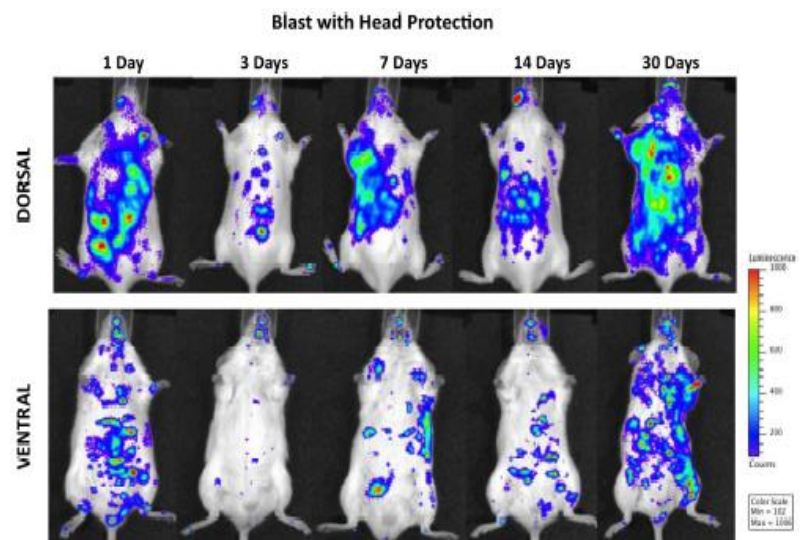
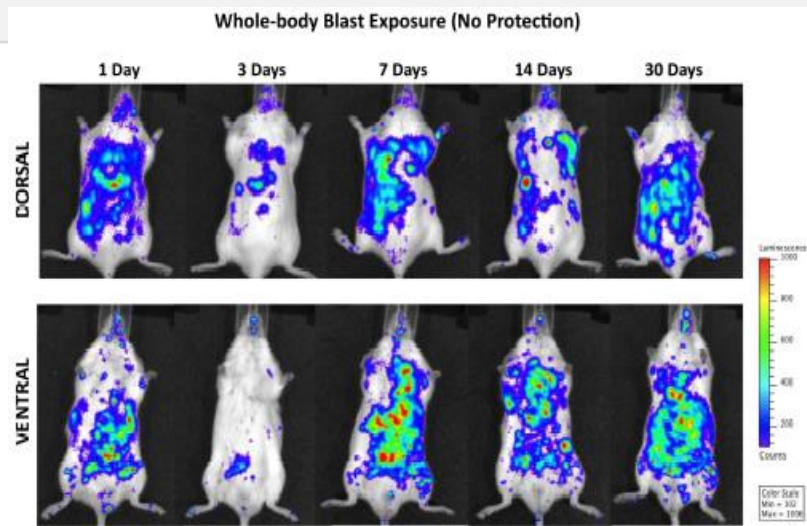
神经毒性



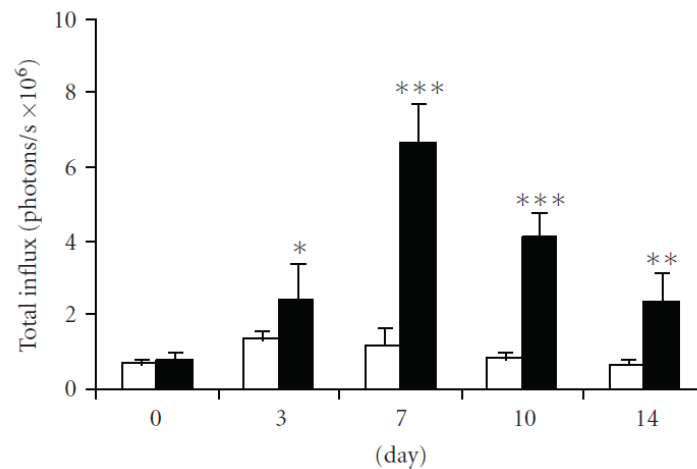
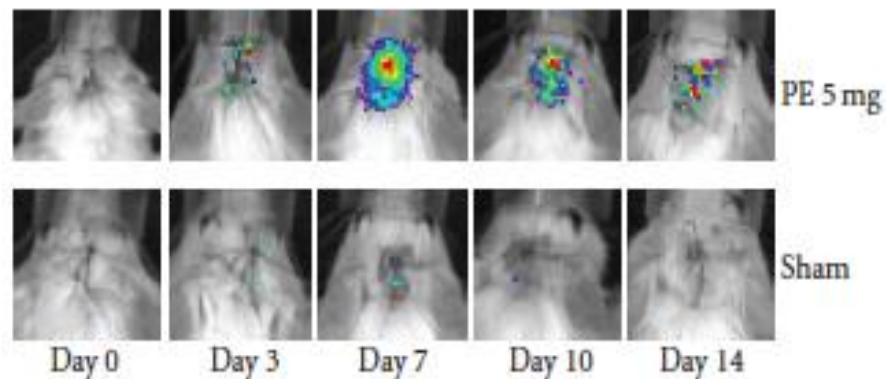
生殖毒性



全身损伤模型&神经毒性

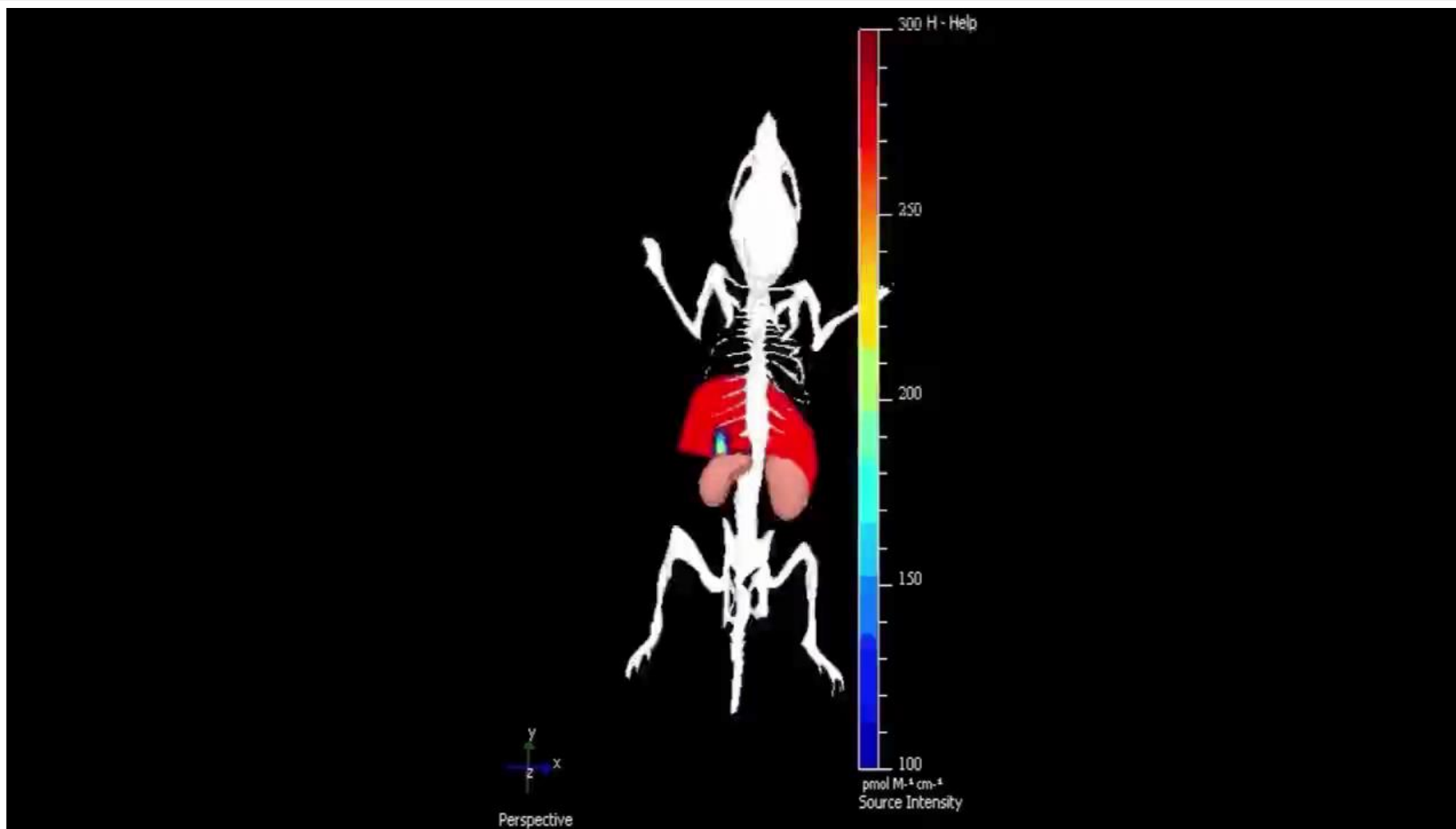


髓过氧化物酶MPO检测过爆中的外伤性脑损伤



颅骨注射聚乙烯模型中，激活NF-kB信号通路，引起的炎症反应。

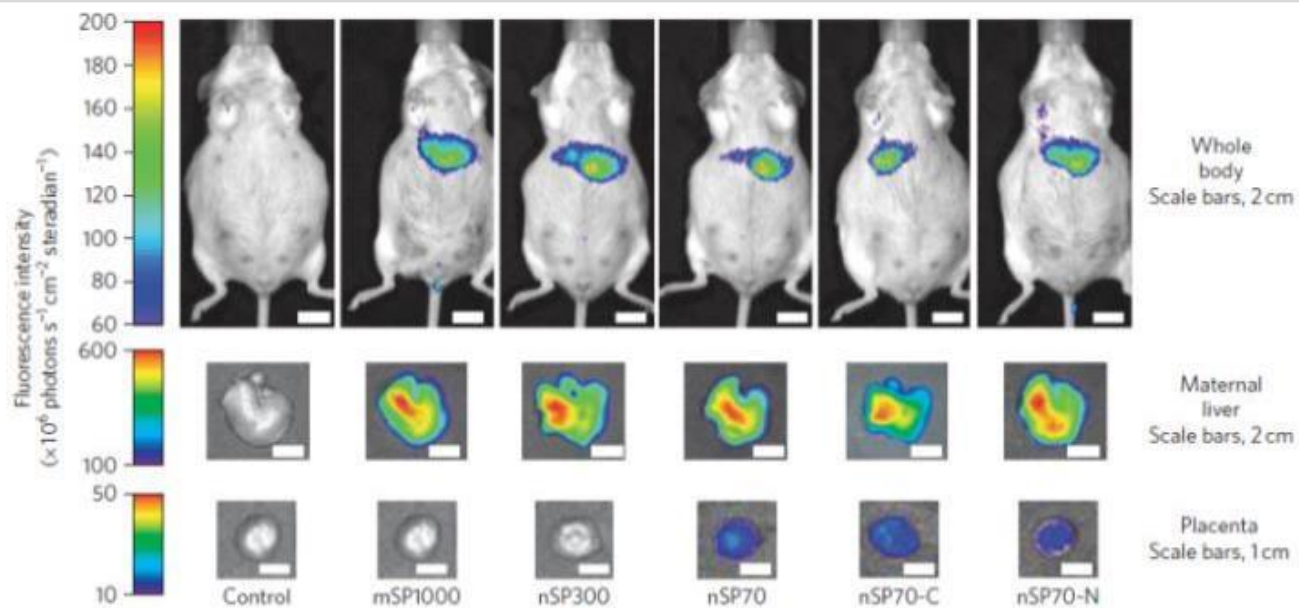
毒性评价——肝脏毒性（肝脏纤维化）



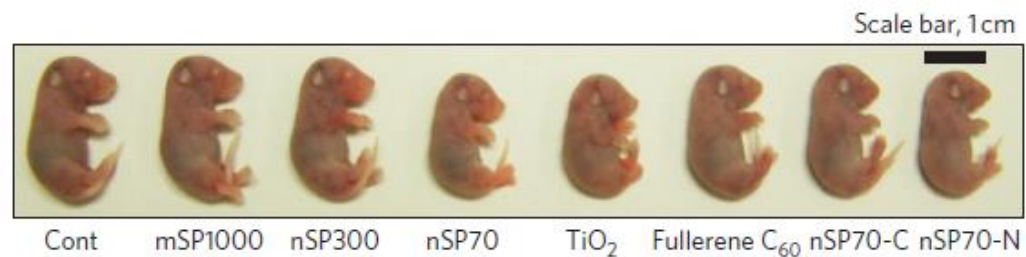
Saimir Luli, et al, Journal of Hepatology,2016

急性肝纤维化模型，C1-3探针检测纤维化

毒性评价——生殖毒性



全部nSPs均可在母鼠肝部产生富集，只有粒径较小的nSP70可以进入胎盘和胎鼠内



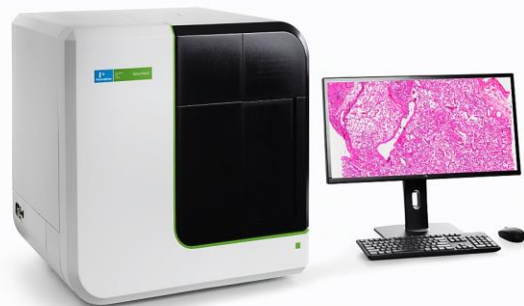
nSP70和TiO₂产生明显毒副作用，导致胎鼠体重明显偏轻；
较大粒径nSPs、COOH/NH₂表面修饰的nSPs、Fullerene均无明显胎毒性

Yamashita K, *et al.* Nature nanotechnology. 2011

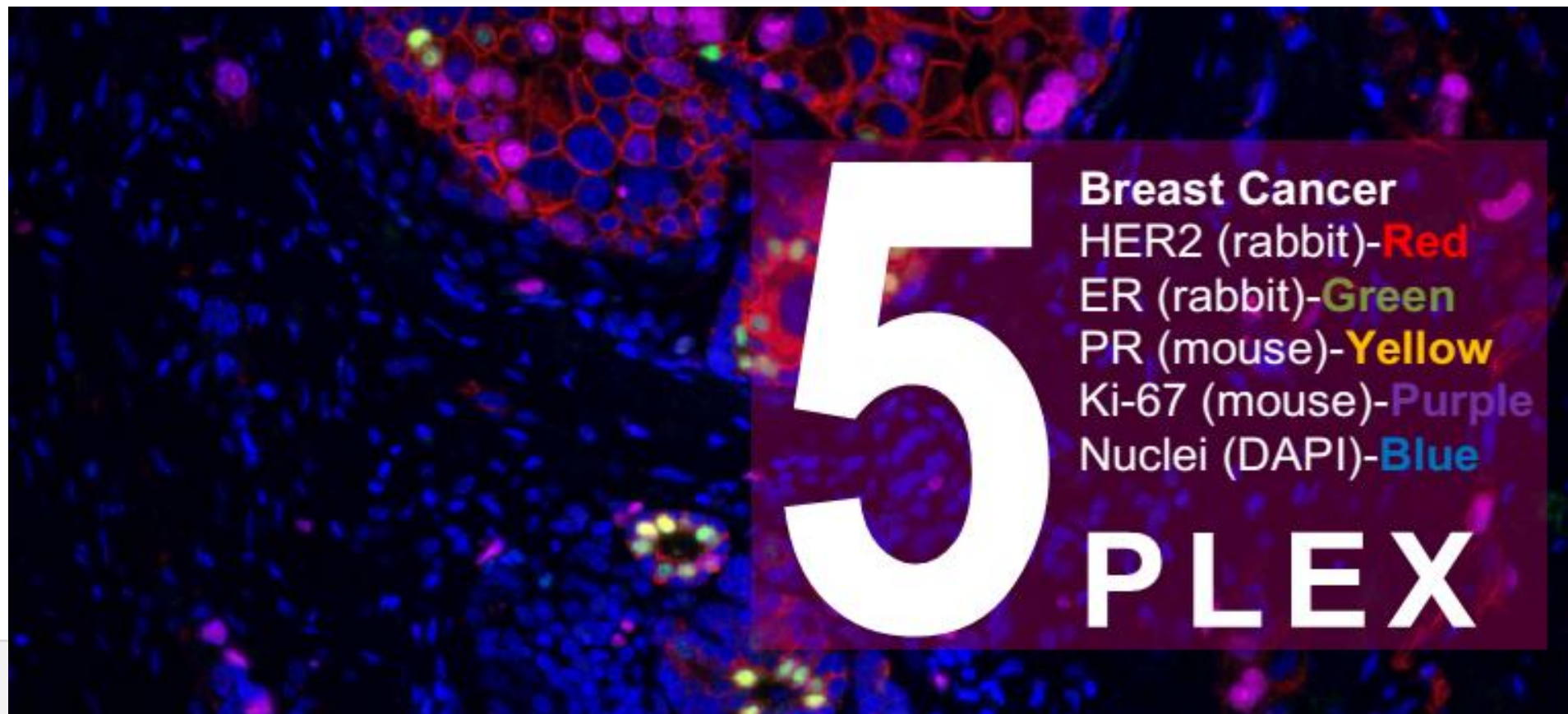
环境污染生物学评价之病理组织多标成像技术



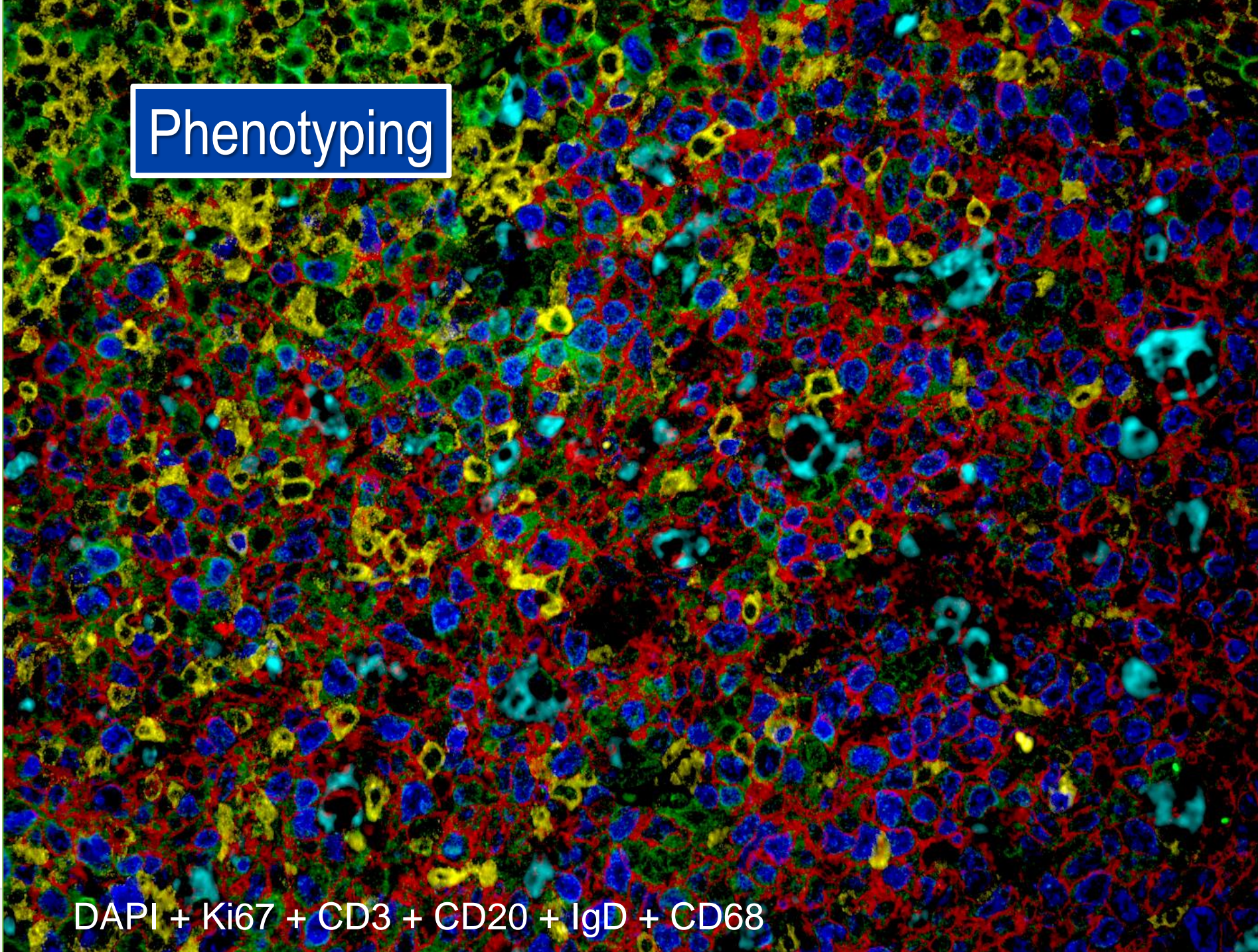
单标+连续切片



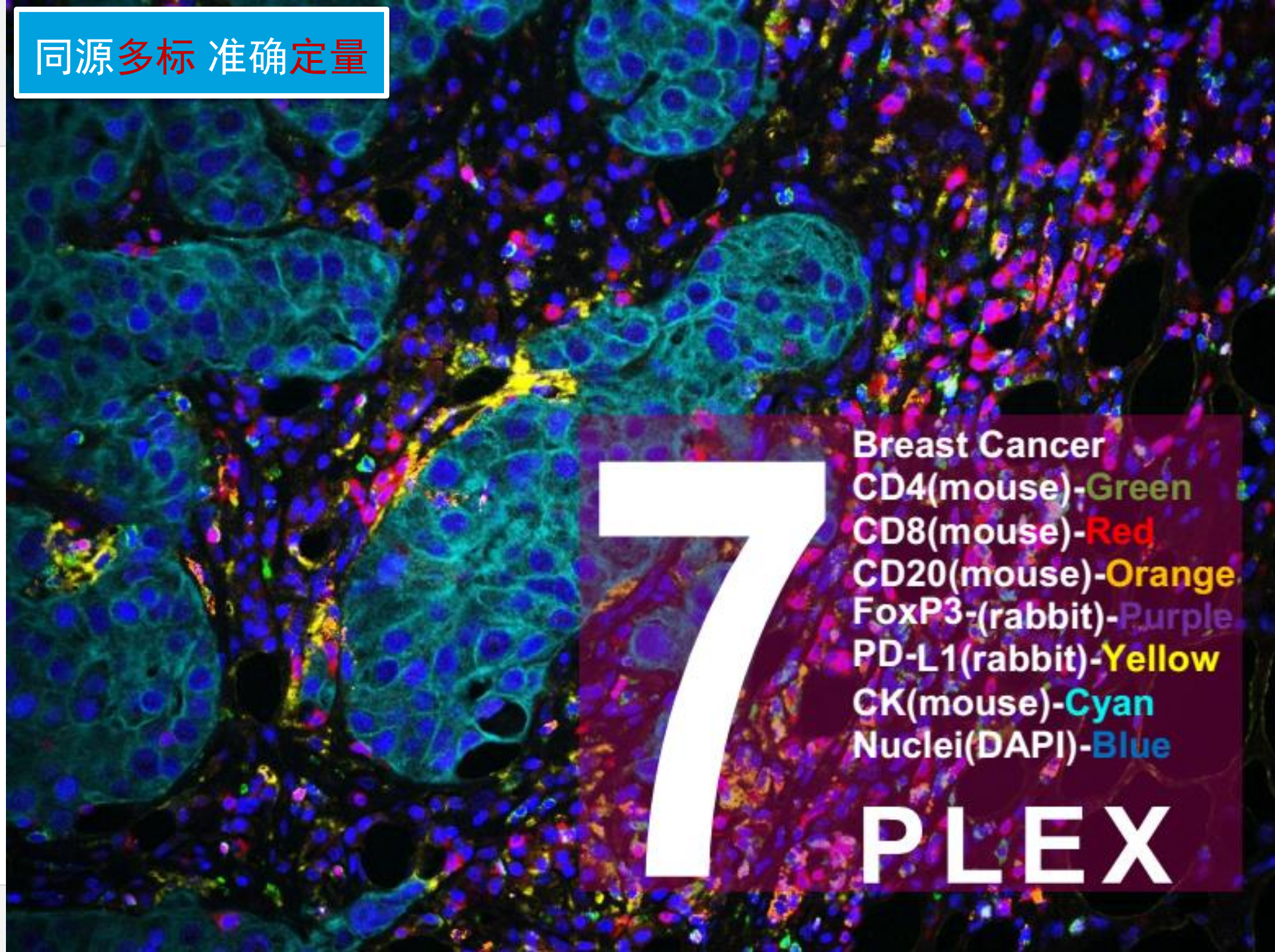
- ✓ 全自动超快速组织切片全景扫描
- ✓ 同源抗体多标染色&多光谱成像
- ✓ 智能化定量分析&海量病理数据挖掘



Phenotyping



DAPI + Ki67 + CD3 + CD20 + IgD + CD68



7

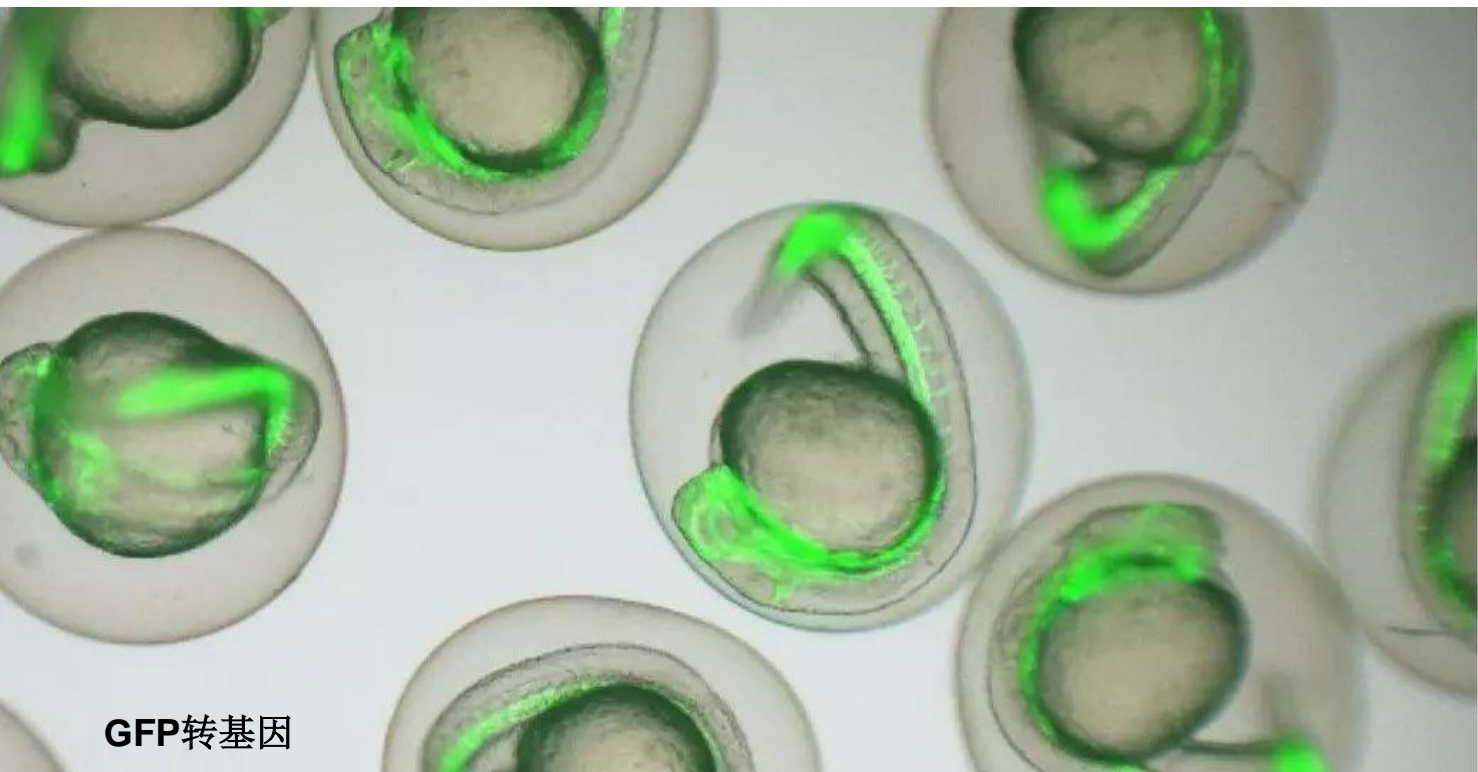
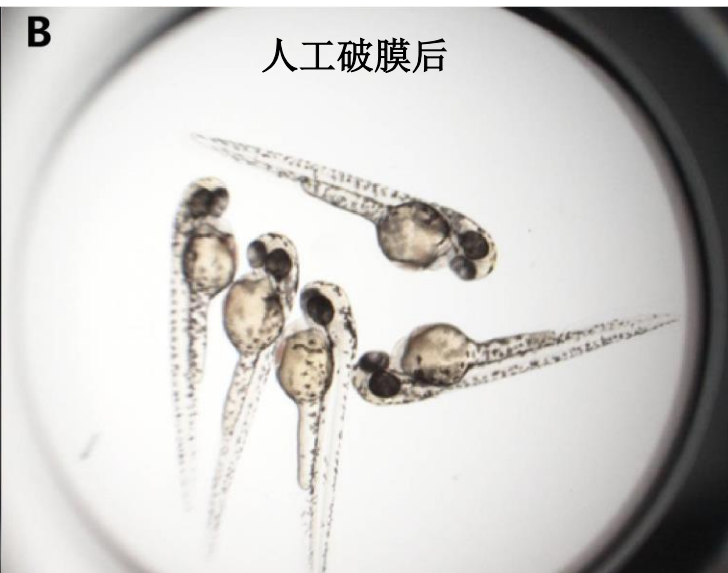
Breast Cancer
CD4(mouse)-Green
CD8(mouse)-Red
CD20(mouse)-Orange
FoxP3-(rabbit)-Purple
PD-L1(rabbit)-Yellow
CK(mouse)-Cyan
Nuclei(DAPI)-Blue

PLEX

斑马鱼：养在96孔板里的动物模型

环境检测与化学品风险评估

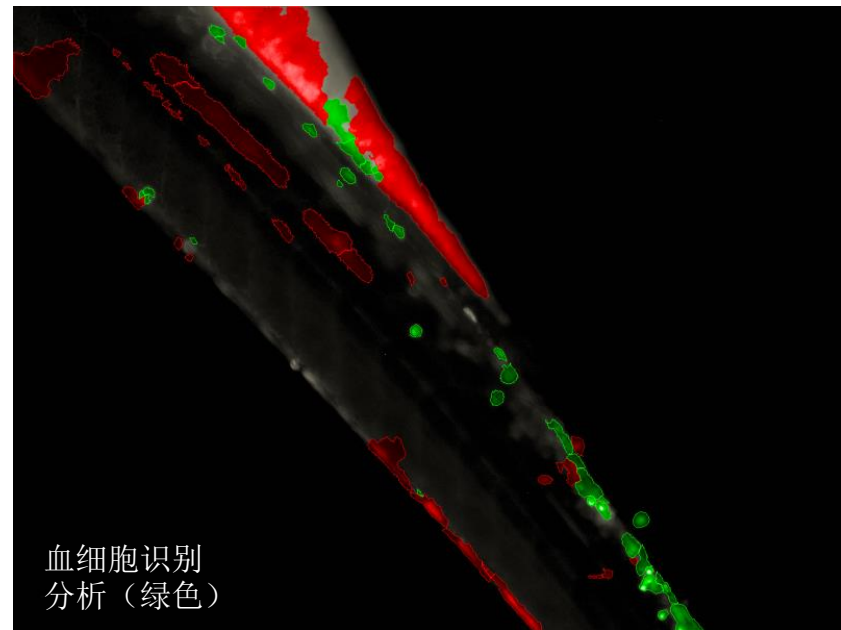
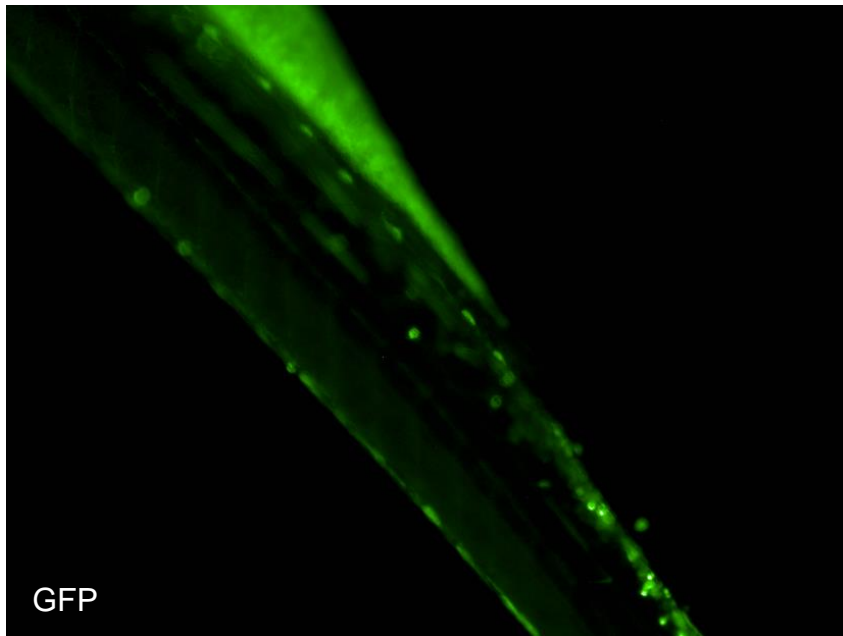
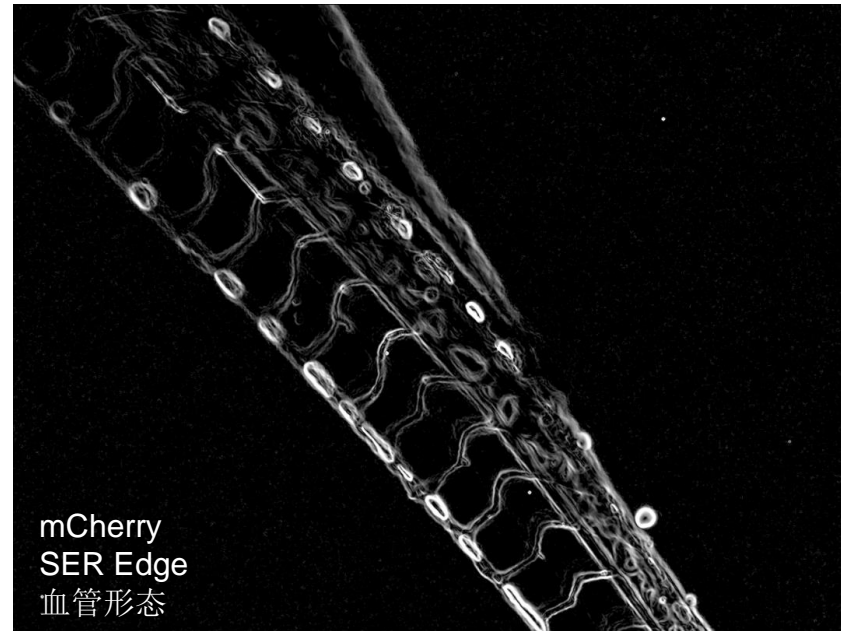
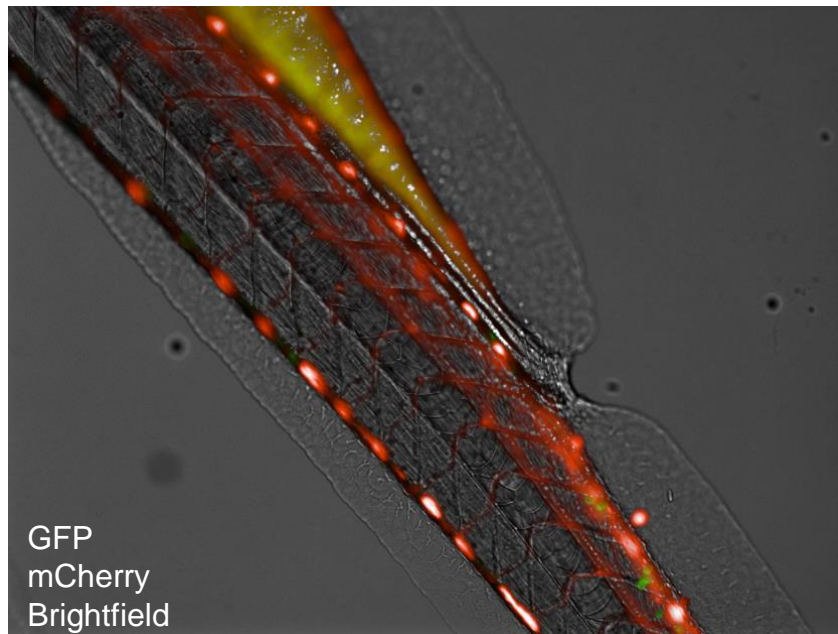
图片来自：上海南方模式动物中心



96孔板中2天的斑马鱼

受精后2天的斑马鱼幼鱼胚胎，体长不到2mm

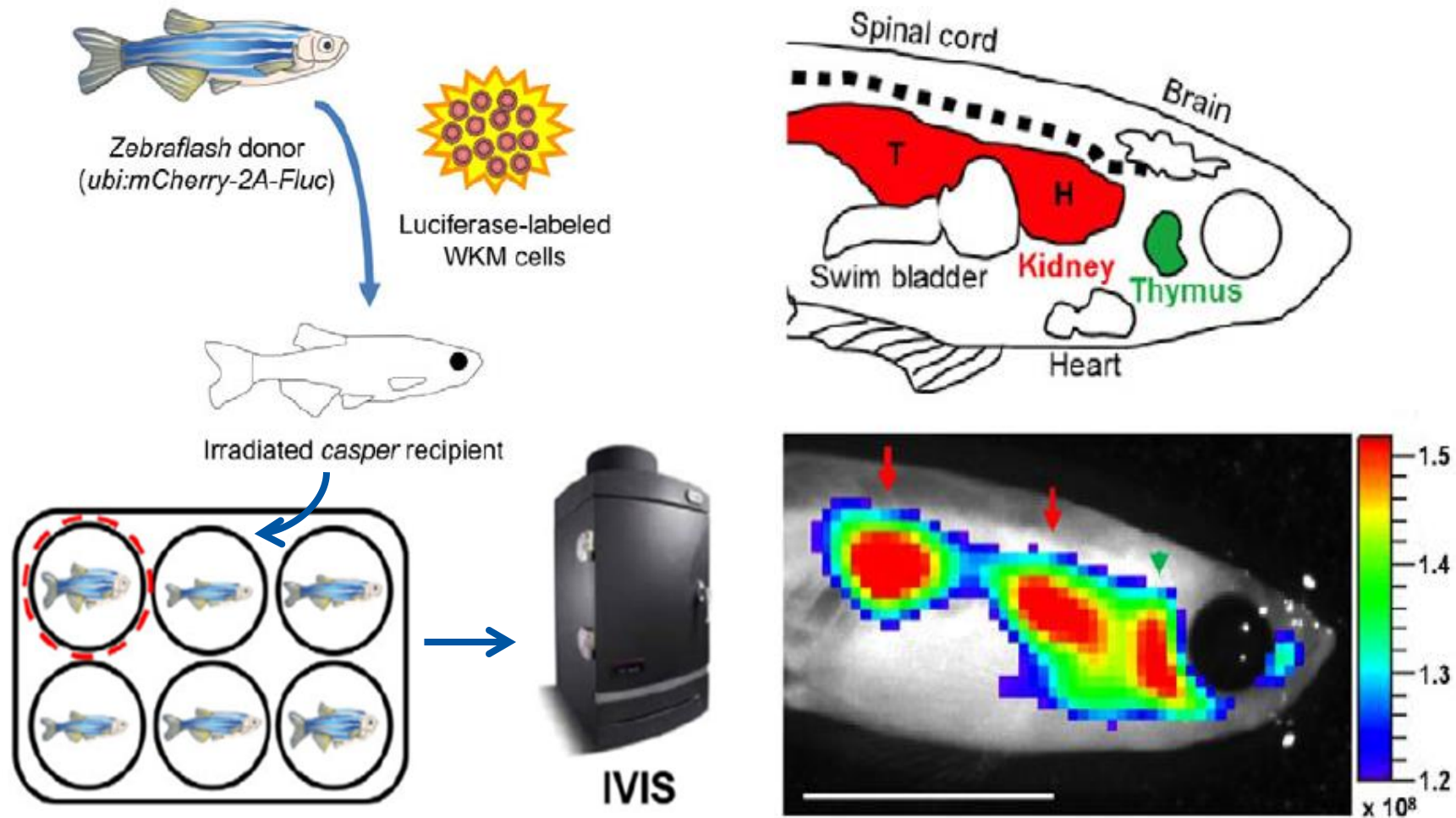
高内涵显微成像分析斑马鱼血管和血细胞



Images are acquired by Operetta CLS 10X Long WD and analyzed by Harmony

zebraflash transgenic lines for *in vivo* bioluminescence imaging of stem cells and regeneration in adult zebrafish

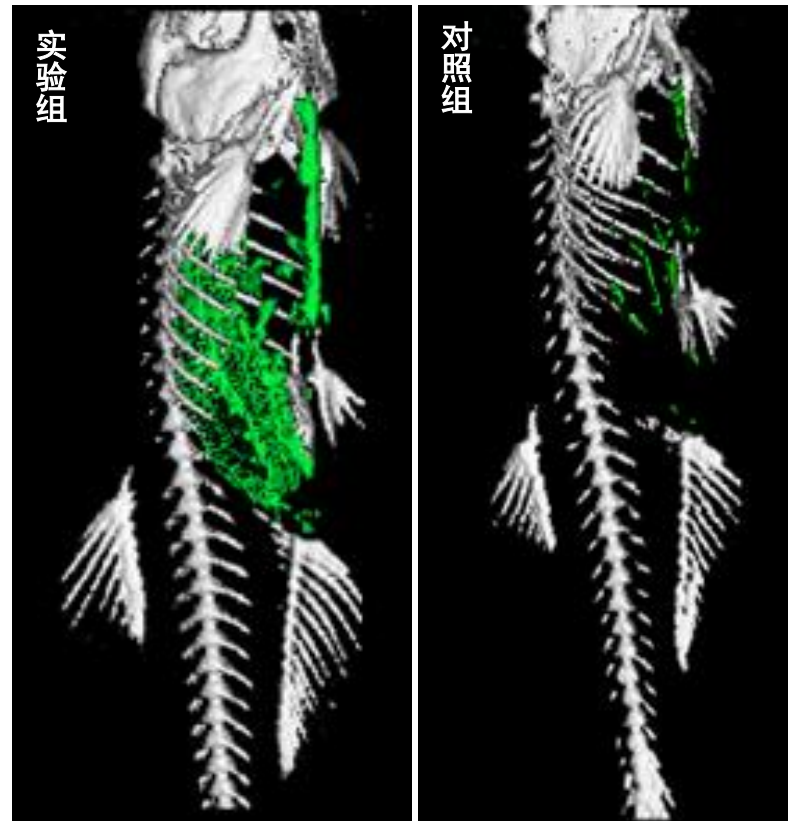
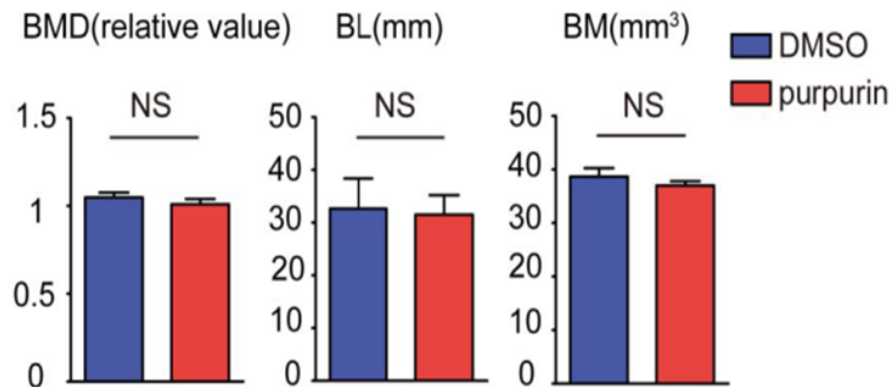
Chen-Hui Chen¹, Ellen Durand², Jinhu Wang¹, Leonard I. Zon² and Kenneth D. Poss^{1,*}



斑马鱼体内脂肪分析

Micro CT 结构成像

斑马鱼骨骼分析



实验组

| Name | MaxVal | MinVal | Mean | Std.Dev. | Vol_mm3 |
|--------------|--------|--------|---------|----------|---------|
| Object_5_sum | 2050 | 1880 | 1984.49 | 46.48 | 5.64 |

对照组

| Name | MaxVal | MinVal | Mean | Std.Dev. | Vol_mm3 |
|----------|--------|--------|---------|----------|---------|
| FAT2_sum | 2050 | 1905 | 2006.71 | 32.72 | 0.44 |

鱼身长: 约4cm 扫描时间: 4min 电压: 80kv 电流100uA 成像视野: 36mm

PerkinElmer 环境毒理整体解决方案

为了更好的生态环境和人类健康

环境污染物分析

无机污染物

- 大气颗粒物中有毒元素分析
- 饮用水中As、Hg、Cd等分析
- 土壤及固体废弃物中重金属分析



PinAAcle AA



FIMS



Ario™ 200



NexION™ 2000 ICP-MS

有机污染物

- 大气中苯系物 (MAHs) 分析
- 水中持久性污染物 (POPs) 和环境激素类物质分析
- 水中杀虫剂、除草剂分析



TurboMatrix HS



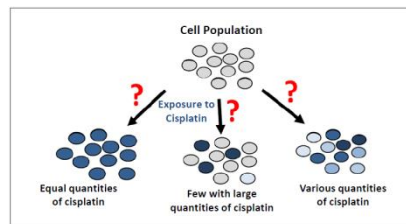
Clarus SQ8 GC/MS



Q Sight™ LC/MS/MS

环境中纳米颗粒分析——单颗粒/单细胞 ICP-MS

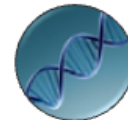
- 单细胞中纳米颗粒的定性定量以及粒径分布表征



生物安全性及毒性评价

致病机制

- 基因畸变
- 代谢毒性
- 免疫毒性
- 生殖毒性

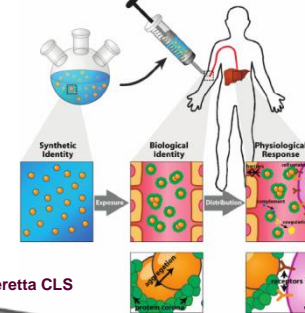


细胞毒性
氧化应激
激素/细胞因子
信号通路

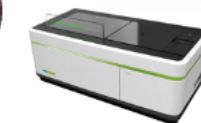


MMD EnSight

纳米颗粒功能分析



微核检测
转位分析
线粒体功能
细胞形态学

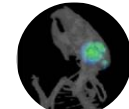


HCS Operetta CLS

功能研究

- 体外检测
- 细胞成像
- 活体成像
- 组织成像

IVIS Spectrum CT



动物整体毒性
生物相容性
新型探针开发
纳米给药系统



Vectra Polarix

原位组织病理
肿瘤微环境
免疫标志物
组织共定位

PerkinElmer开创性的开放式共建实验室



先进

开放

创新

合作

对外服务

市场推广

技术培训

应用开发



生化工程国家重点实验室-珀金埃尔默共建实验室实景展示



实验室仪器预约系统



机时服务

预存款送机时

老用户赠机时

好文章奖机时

技术服务

病理切片多标服务

活体成像实验服务

高内涵药物筛选服务

分子水平药筛服务

四名全职技术人员，多名兼职技术专家，确保服务专业而到位。

会议、培训、参观现场





实验室介绍

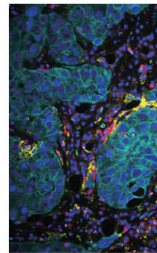
转化医学是我国十三是搭起基础研究和临床应用作为一家全球型的以创新领域的技术供应商。在进技术和仪器平台。为了触和了解这些先进的技术方方案。更好地支持和培训后在上海和北京开创性地服务。成为开放技术平台中心。

2014年3月。珀金埃尔成立了全球最顶级的“复共建小动物活体影像示范-细胞-小动物-组织成像实验室配备两名全职工作持。目前使用时率接近和文献。

2017年9月。珀金埃尔立了“生化工国家重点程共建实验室”。代表着台。该实验室占地100平方配备10台从分子到细胞、分为细胞实验室、病理与个功能区。

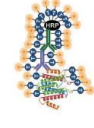
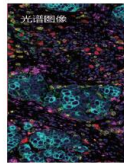


PerkinElmer



- 组织切片多色标记
- 多光谱组织成像
- 智能化病理定量
- 高通量批量分析

专业的组织微环境



实验指南-1

肿瘤研究技术

- 细胞增殖
 - 增殖过程评价
 - 细胞分裂及分子机理
 - 细胞周期及分子机理

| | |
|------|---|
| 高内涵 | ✓ |
| 酶标 | ✓ |
| 组织成像 | ✓ |
| 小动物 | ✓ |

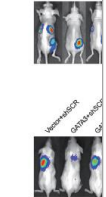
分子水平实验

- 普通细胞
- 肿瘤细胞
- 肿瘤细胞团
- 逃避免疫监控
- 内部坏死/空心化
- 血管新生
- 侵袭基质层和血管壁
- 多组织和脏器的转移

实验指南-2

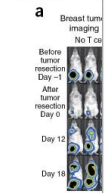
小动物活体

常用于肿瘤通过转基因技术用。2. 通过外映肿瘤的发展变究。2. 抗肿瘤



癌症分子机节失常促进Si et al., Ca

研究实例2. 1



肿瘤免疫相有生物活性肿瘤附近或增和分散抑



生化工国家重点实验室-珀金埃尔默转化医学工程共建实验室
地址：北京市海淀区中关村二环路11号中科院过程工程研究所生化工程研楼201室
联系人：马文瑞 15117950406 白宗良 13811586393



复旦大学基础医学院-珀金埃尔默转化医学共建实验室
地址：上海市徐汇区东安路131号上海医学院东一号楼354房
联系人：郭晓 17721121417



欲预约相关实验，请扫描二维码

珀金埃尔默企业管理(上海)有限公司

上海总公司
地址：上海浦东新区张江高科
张江高科东环路1670号
电话：+86 21-6064 5888
传真：+86 21-6064 5999
邮编：201203

北京分公司
地址：北京朝阳区望京桥路14号附
望京工业基地2号楼东南侧单元
电话：+86 10 8434 8999
传真：+86 10 8434 8988
邮编：100075

成都分公司
地址：成都高新西区百草大道5号
汇通中心B座12楼
电话：+86 28-8785 7220
传真：+86 28-8785 7221
邮编：610016

武汉分公司
地址：武汉市武昌区临江大道96号
武汉方舱中心东楼1008-1009室
电话：+86 27-8891 3055
传真：+86 27-8891 3380
邮编：430062

西安分公司
地址：陕西省西安市雁塔区
二环南路南段雁鸣园广场11层
电话：+86 029-8129 2671
传真：+86 029-8129 2126
邮编：710065

广州分公司
地址：广州市海珠区芳村大道
广市美利号信义会馆12号
电话：+86 20-3789 1888
传真：+86 20-3789 1899
邮编：510370

中文网址: www.perkinelmer.com 客户服务热线: 800 820 5946 400 820 5946

要获取我们位于全球的各个办公室的完整列表，请访问 <http://www.perkinelmer.com/AboutUs/ContactUs/ContactUs/>

版权所有 © 2018, PerkinElmer Inc. 保留所有权利。PerkinElmer 是 PerkinElmer Inc. 的注册商标。其它所有名称均为各自所有者或所有者的财产。

本资料中的信息，说明和技术规格如有变更，恕不另行通知。





Thank you! Any Questions?

冯起

DAS LST 技术经理

qi.feng@perkinelmer.com

18611637637

