

# 环境毒理整体解决方案

生物安全性及毒性评价

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2017年10月27日



HUMAN HEALTH • ENVIRONMENTAL HEALTH

# 环境污染影响人类健康

无机污染物

有机污染物

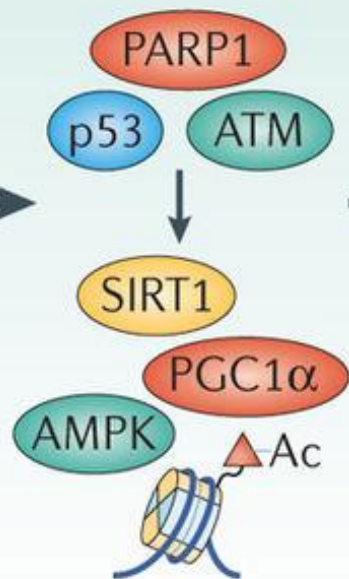
颗粒污染物

放射性污染

Nuclear DNA damage



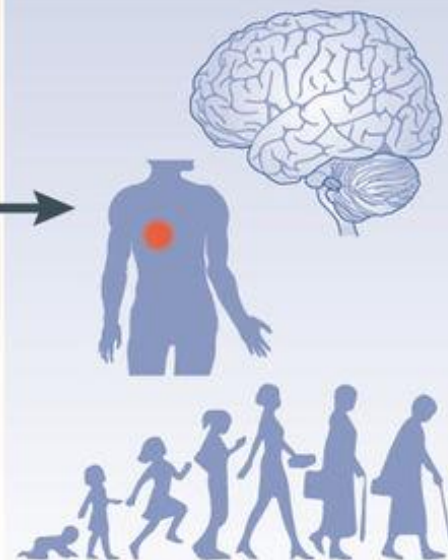
Signal transduction



Mitochondrial dysfunction



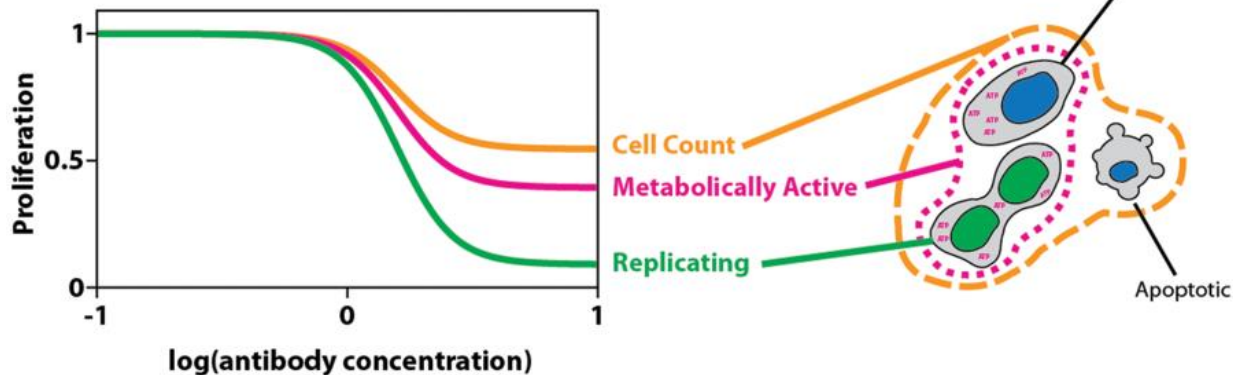
Neurodegeneration, cancer and ageing







# 细胞增殖 细胞毒性 细胞死亡

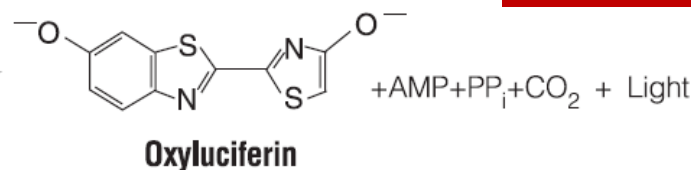
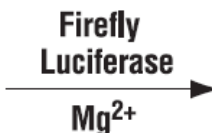
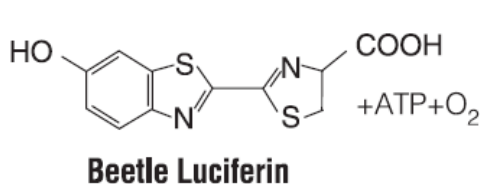
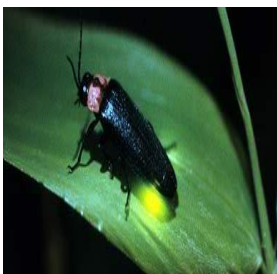


检测技术	主要实验方法	灵敏度	背景噪声	特异性	实验周期
光吸收	MTT类检测 (490 nm) CCK8 (WST-8) 检测 (450 nm) LDH检测 (500 nm) SRB检测 (540 nm)	低	中	中等	1-2小时
荧光强度	阿尔玛蓝 (Alamar Blue) (530/590 nm) Calcein-AM (活细胞荧光染料)	中等	高	中等	>2小时
化学发光法	ATP检测法ATPLite 细胞色素P450酶活检测	高	低	高	< 20分钟
时间分辨荧光	DNA片段化检测 (DELFI DNA fragmentation) DNA复制检测 (DELFI BrdU Proliferation) 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

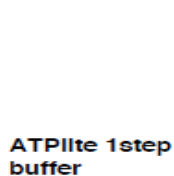
# ATPlite 1step™ 细胞活性检测



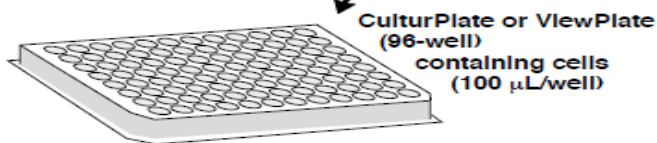
540~600nm



- 1** Preparation of substrate solution:
- 10 mL kit : Add 10 mL buffer/vial
  - 100 mL kit : Add 10 mL buffer/vial
  - 1,000 mL kit: Add 250 mL buffer/vial

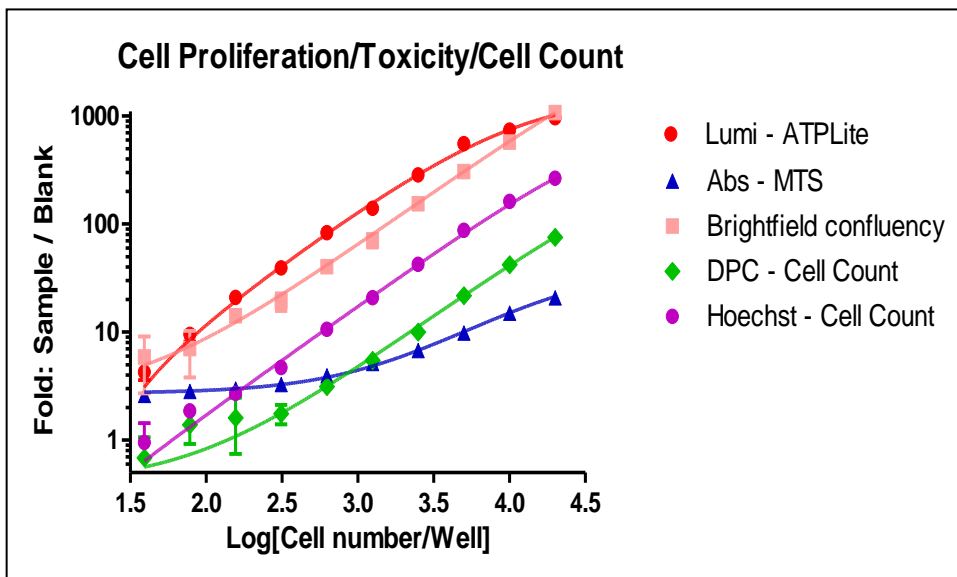


Add 100 μL/well **2**



Seal and mix

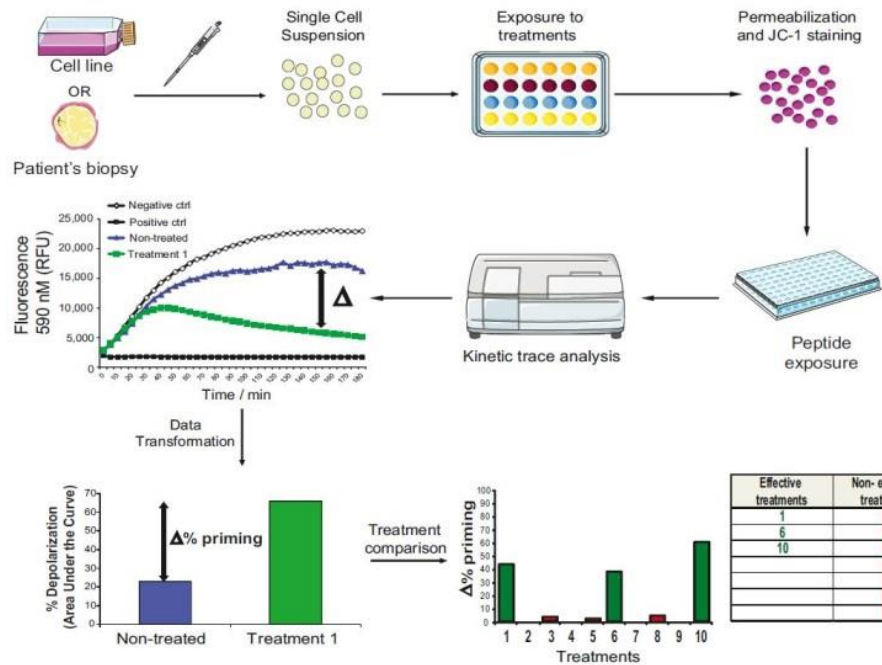
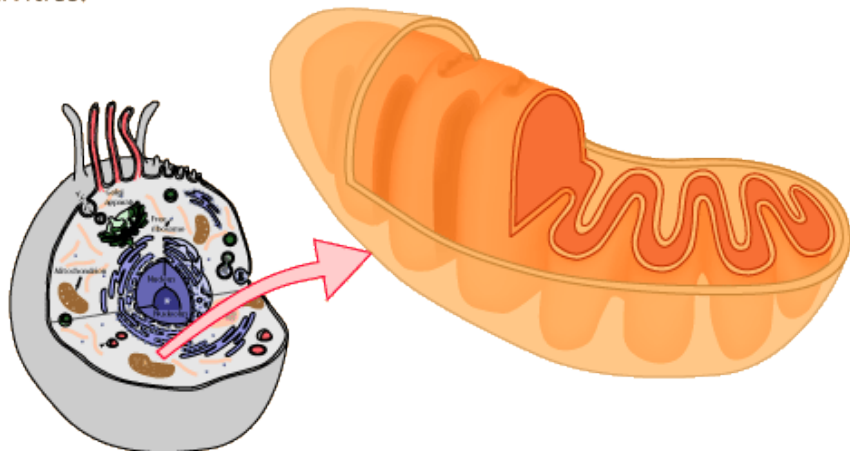
**3** Measure luminescence



- ❖ 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

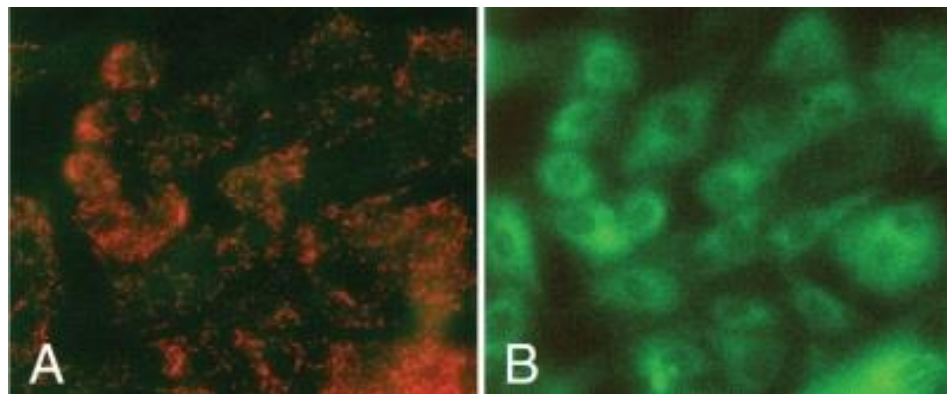
# 线粒体毒性

The cell's energy factories, the mitochondria manufacture ATP to fuel all of life's activities.



[Ref: Paulina Marzec *et. al.* Cell (2015) 160, 913–927, 26.]

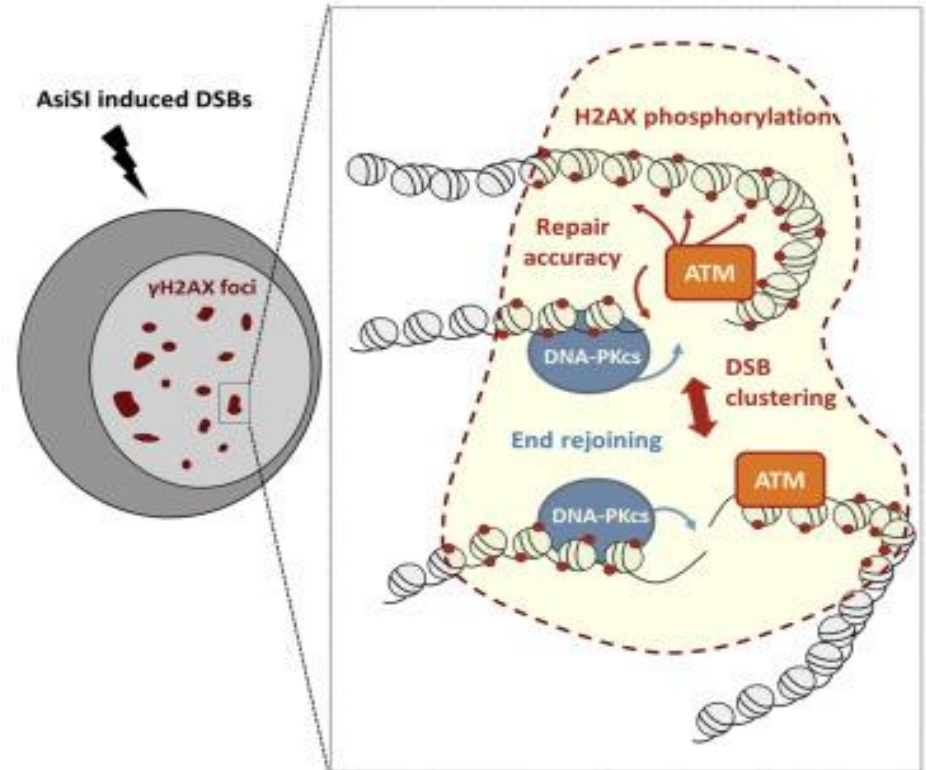
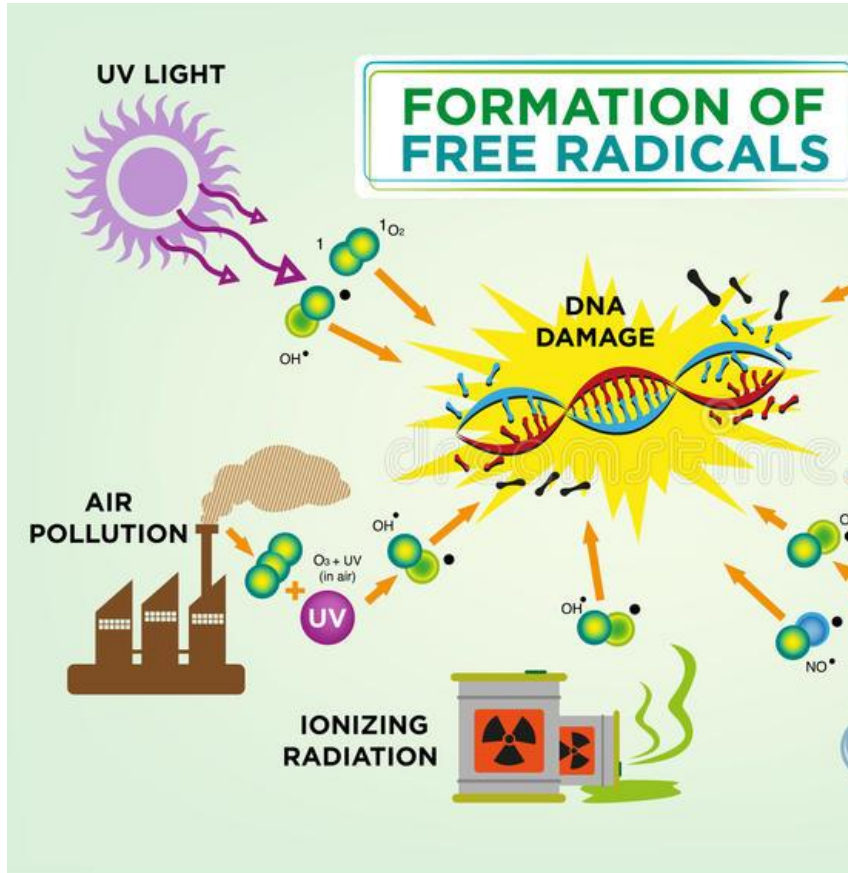
- ATP含量检测 (酶标仪)
- 线粒体氧化呼吸检测 (酶标仪)
- 线粒体膜电位检测 (酶标仪/高内涵)
- ROS含量检测 (酶标仪/高内涵)
- 线粒体形态检测 (高内涵)



A:膜电位高：聚合体红色通道成像

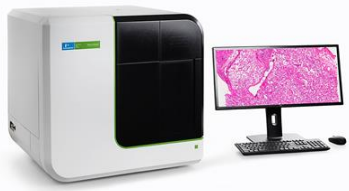
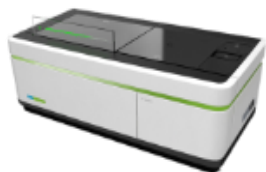
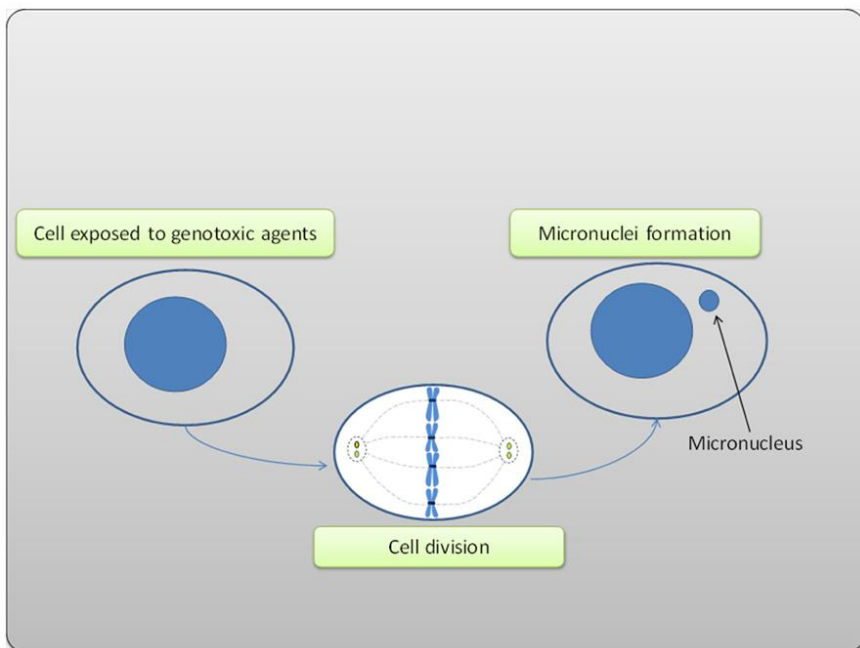
B:膜电位低：单体绿色通道成像

# 组蛋白H2AX磷酸化抗体特异检测双链DNA断裂



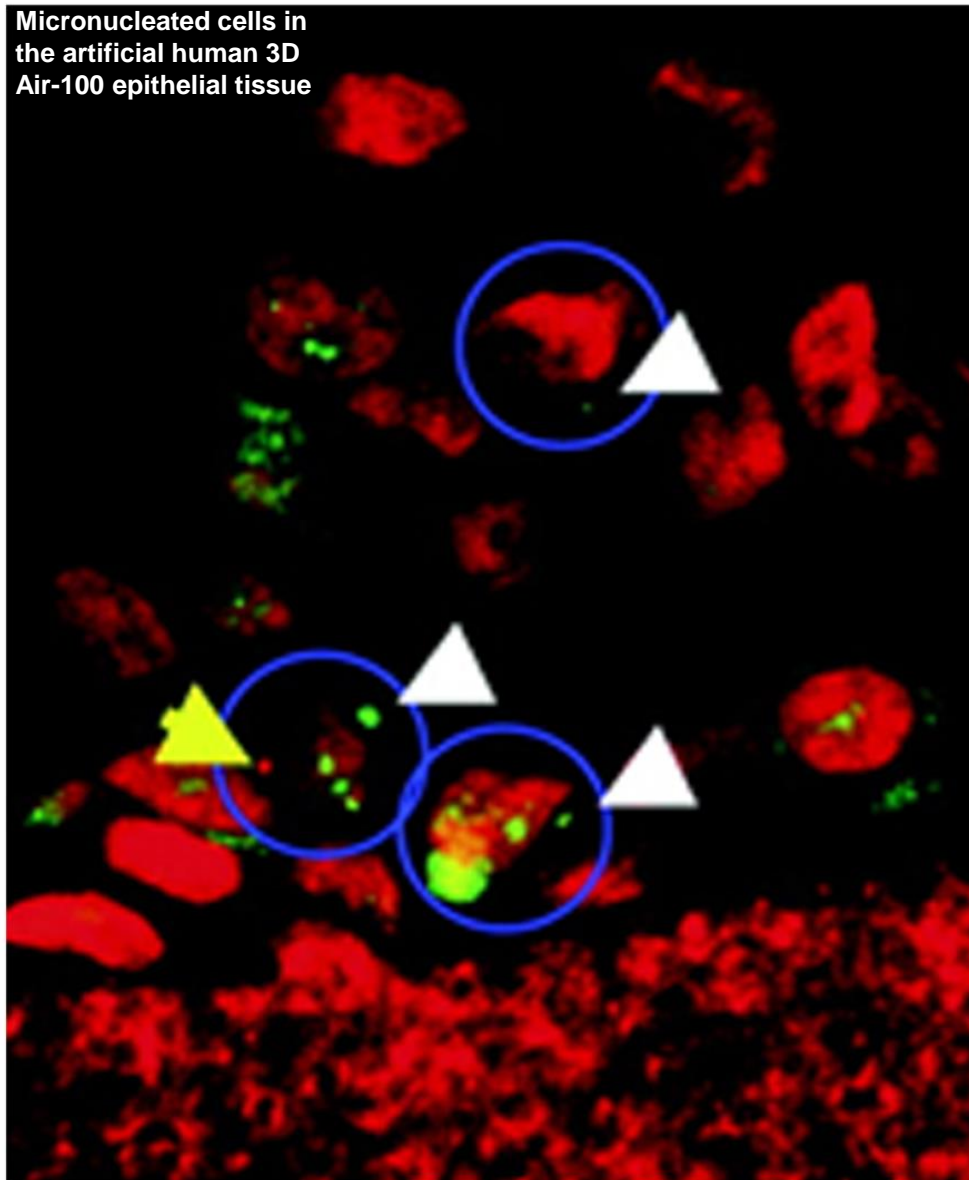


# 遗传毒性染色体缺失 微核形态学检测分析



高内涵成像/组织定量病理成像

Micronucleated cells in  
the artificial human 3D  
Air-100 epithelial tissue

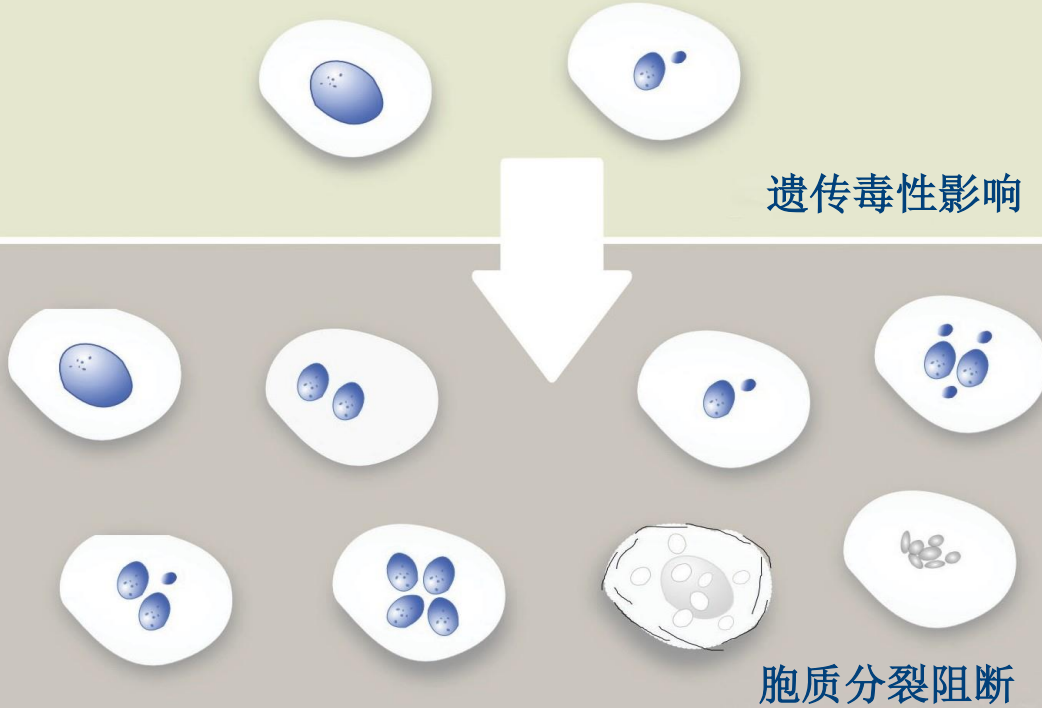


红色 PI染色；绿色 H2AX抗体染色  
白色箭头：H2AX阳性微核；黄色箭头：H2AX阴性微核



# 微核检测的精密评分算法

遗传毒性影响

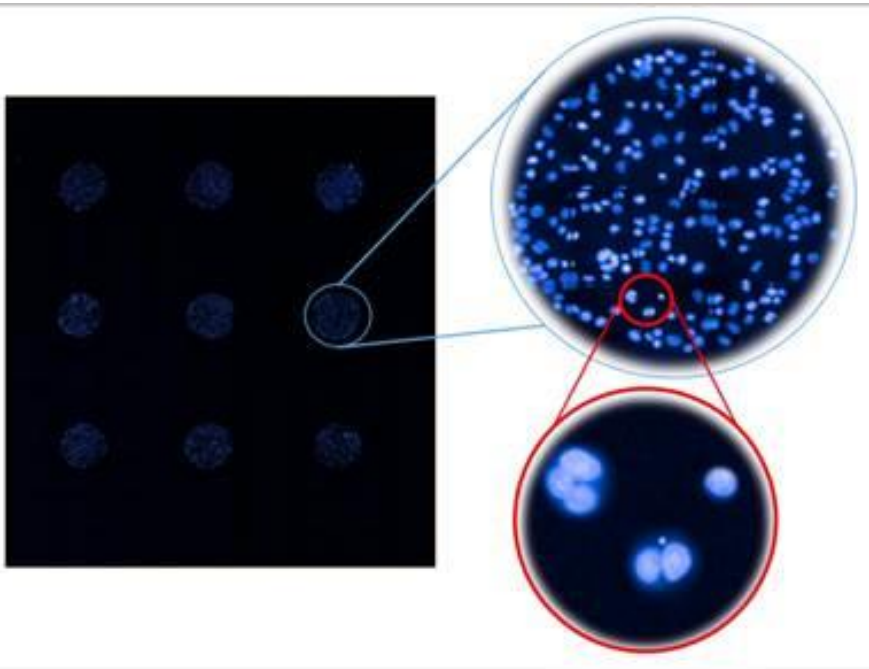
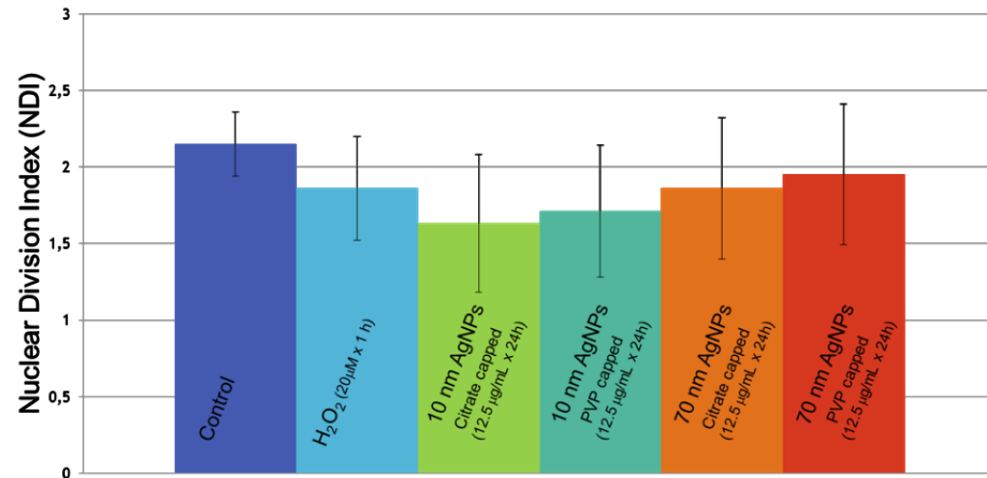


## 主要特点

- 新型 Acapella® 微核频数评分算法
- 单核细胞、双核细胞和多核细胞的鉴定
- 参数调整过程中的图形反馈
- 利用高内涵筛选系统进行固定细胞的自动化图像采集

## Genotoxic effect induced by AgNPs in human B lymphocyte cell line

NDI



# 新一代均相免洗高灵敏AlphaLISA检测方法

## ALPHA (Amplified Luminescent Proximity Homogenous Assay)

❖ 生物标志物检测  
ER、AR、PD-L1

❖ 信号通路研究  
Kinase/GPCR/ Epigenetics

❖ 污染物及残留物监测  
黄曲霉素/氯霉素/凝集素/转基因蛋白

❖ 新型生物传感器开发





# A platform for development of novel biosensors by configuring allosteric transcription factor recognition with amplified luminescent proximity homogeneous assay

Shanshan Li,<sup>a</sup> Li Zhou,<sup>b</sup> Yongpeng Yao,<sup>a</sup> Keqiang Fan,<sup>a</sup> Zilong Li,<sup>a</sup> Lixin Zhang,<sup>ab</sup> Weishan Wang,<sup>\*a</sup> and Keqian Yang<sup>\*a</sup> *Chem. Commun.*, 2017, 53, 99-102

## Recognition elements (aTFs)

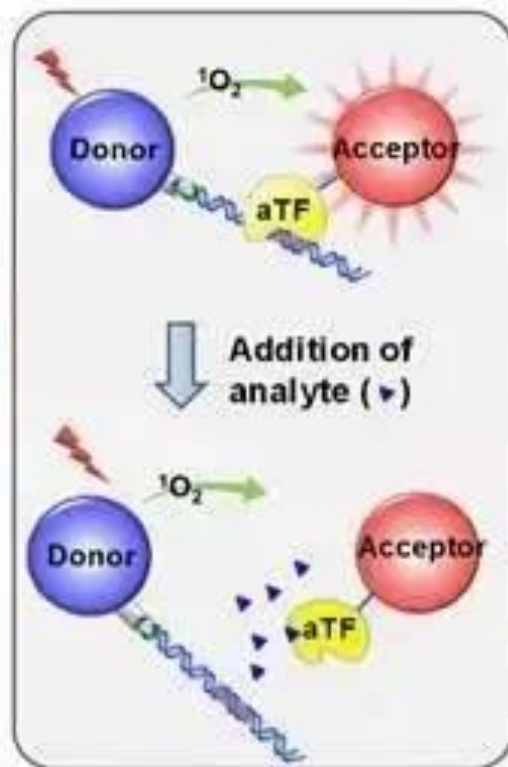
Clinical diagnose  
HucR, AguR, BepR,....

Environmental monitoring  
MerR, ArsR, CadC,....

Food safety detection  
TetR, MphR, PmeR,....

More aTFs

## Biosensing platform



微生物所杨克迁课题组基于多年来对原核生物别构转录因子 (allosteric transcription factor, aTF) 调控机制的认识, 首次将原核生物 aTF 在体外作为识别元件结合, 使用Alpha技术构建感应化学小分子的检测平台。该平台利用Alpha技术和巧妙的实验设计, 针对**临床标志物尿酸和抗生素残留土霉素**的检测, 其适应性更强, 也是目前最灵敏的检测方法。

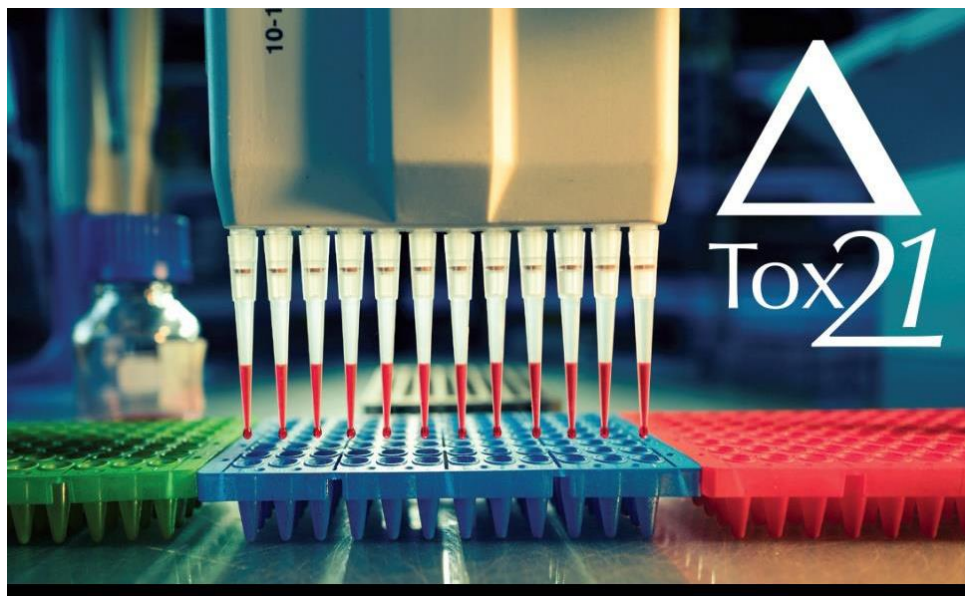


# Toxicology in the 21st Century

## 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.





# NIH NCATS 高通量筛选平台

## Optimization of Instrumentation can Improve Assay Results

**NIH** National Center  
for Advancing  
Translational Sciences



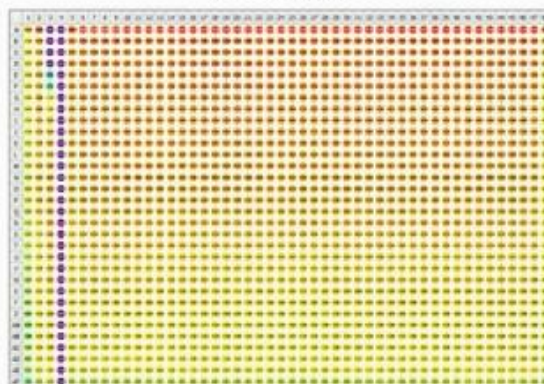
**Nathan P. Coussens** 博士  
高级应用科学家

**Assay Guidance Manual**  
科学副编辑

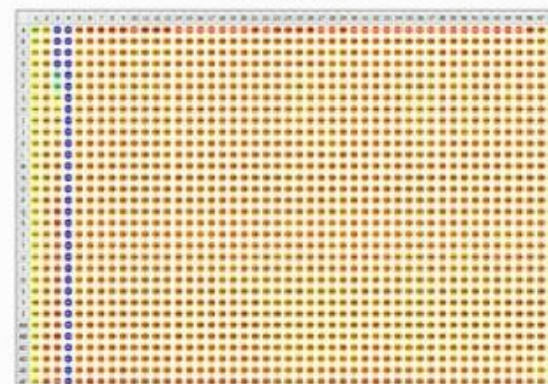
$$Z'\text{-Factor} = 1 - \frac{(3\sigma_+ + 3\sigma_-)}{|\mu_+ - \mu_-|}$$

$$\text{Coefficient of Variation} = \%CV = \frac{\sigma}{\mu} \times 100$$

**%CV = 9.6**  
**Z' = 0.68**



**%CV = 2.5**  
**Z' = 0.90**



Yasgar A., Jadhav A., Simeonov A., Coussens N.P., **AlphaScreen-Based Assays: Ultra-High-Throughput Screening for Small-Molecule Inhibitors of Challenging Enzymes and Protein-Protein Interactions.** *Methods Mol Biol.* 2016;1439:77-98.

**NIH** National Center  
for Advancing  
Translational Sciences

优化后，我将变异系数 CV 从 9.6% 降至 2.5%



# Identifying Environmental Chemicals as Agonists of the Androgen Receptor by Using a Quantitative High-throughput Screening Platform

Volume 385, 15 June 2017, Pages 48-58

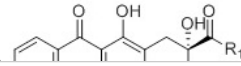


EnVision

AR-bla

*qHTS of AR reporter gene assay in Vitro*  
*Imaging based GFP-AR translocation assay and analysis by HCS*

9 11 13 15 17 19 21 23 25



AR-bla

○ N/A  
 ● > 2.0  
 ○ 2.0



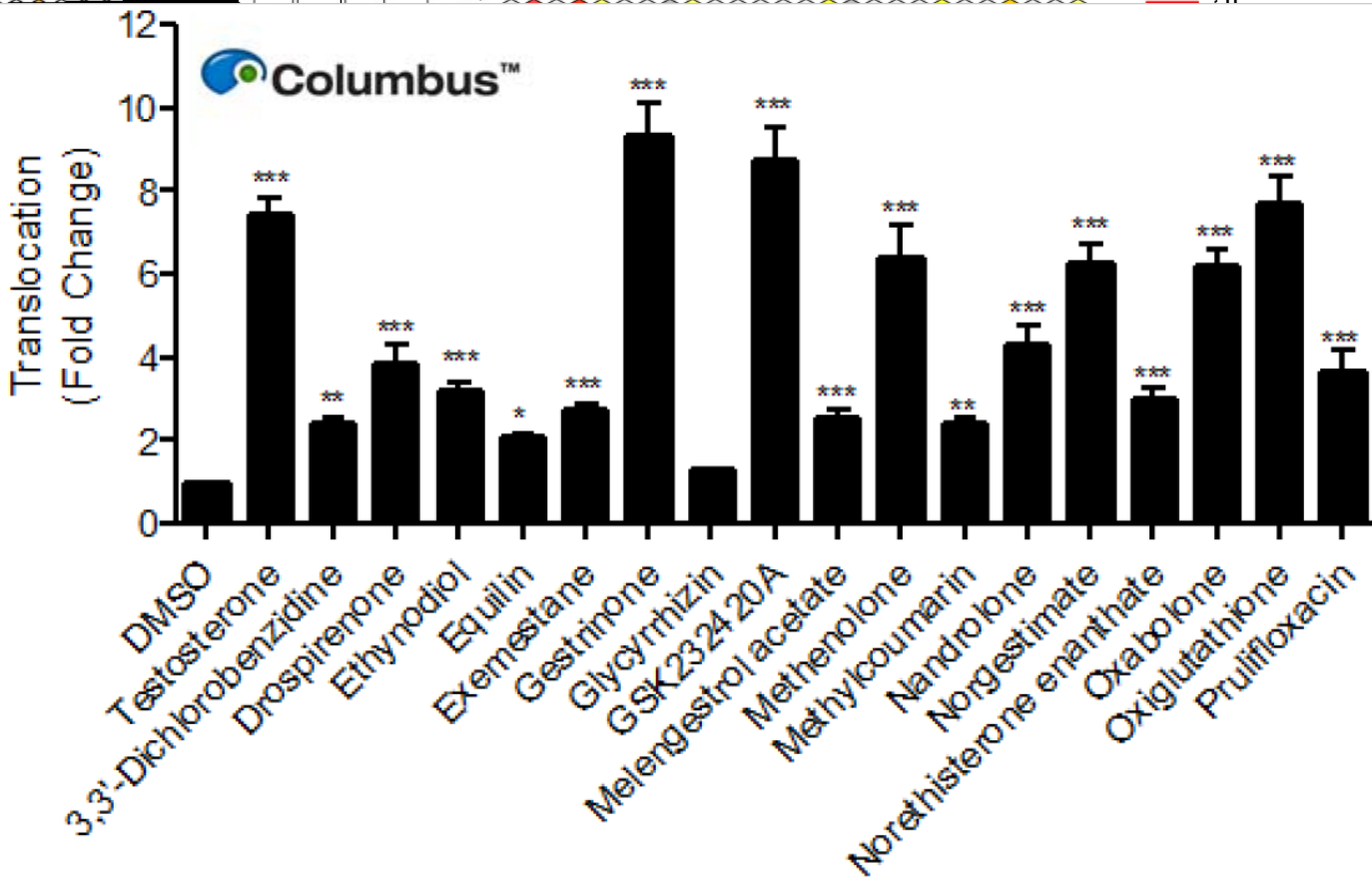
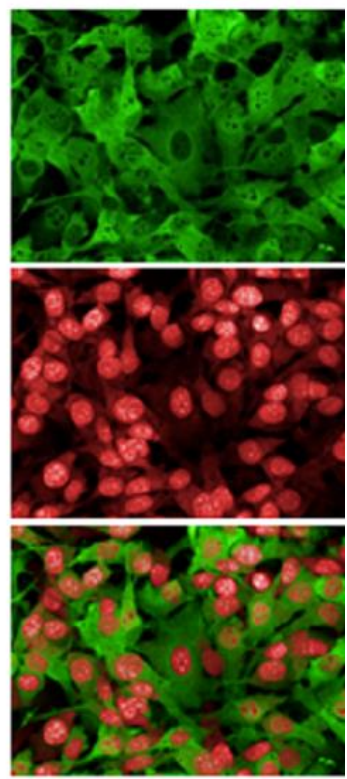
Opera

DMSO

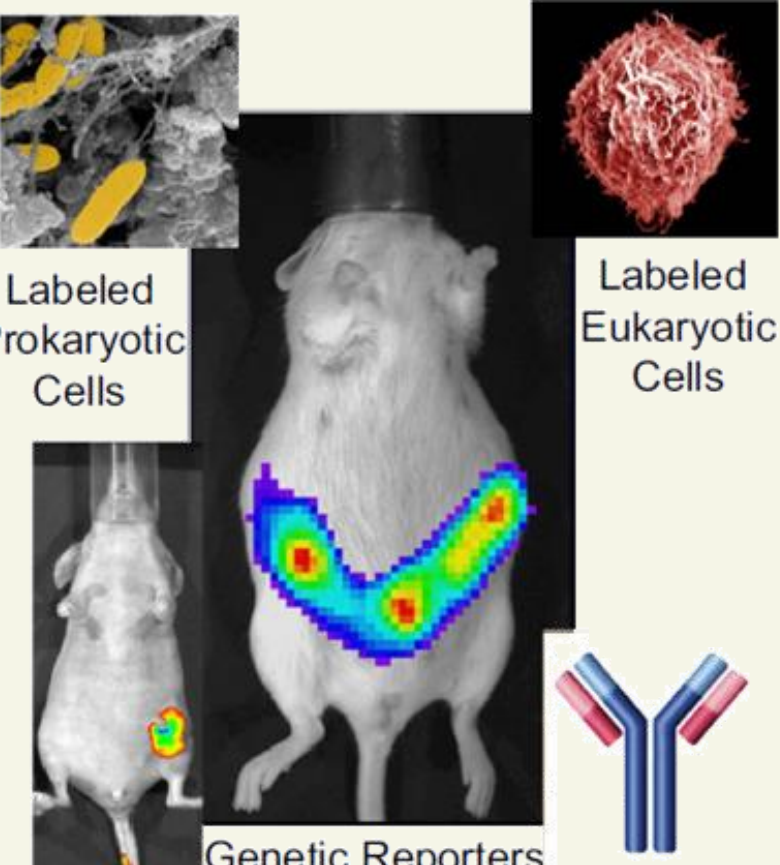
GFP-AR

Nuclear

Mixed



# 小动物活体成像解决方案



Labeled Prokaryotic Cells

Labeled Eukaryotic Cells

Genetic Reporters

Smart Probes

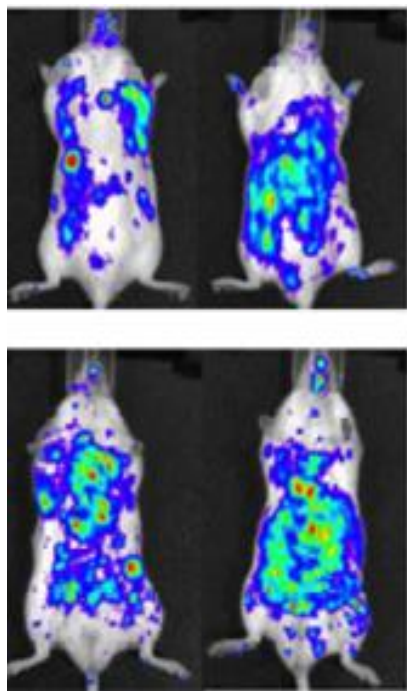
Labeled Proteins



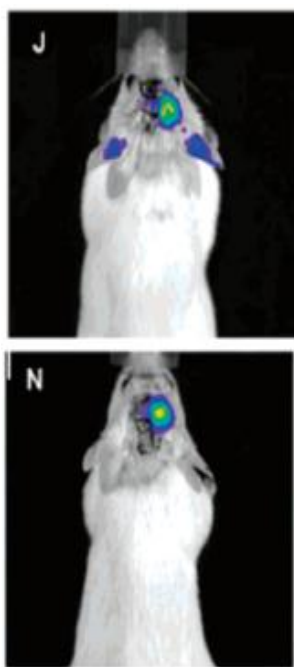


# 动物活性水平毒性评价

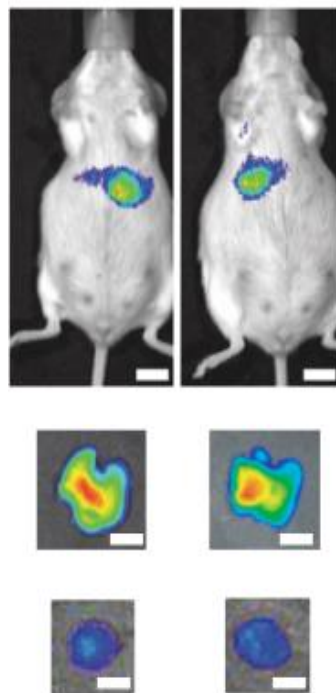
## 全身毒性



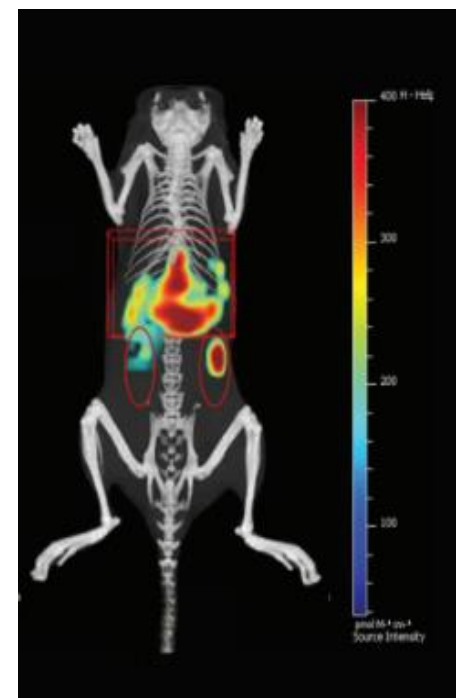
## 神经毒性



## 生殖毒性



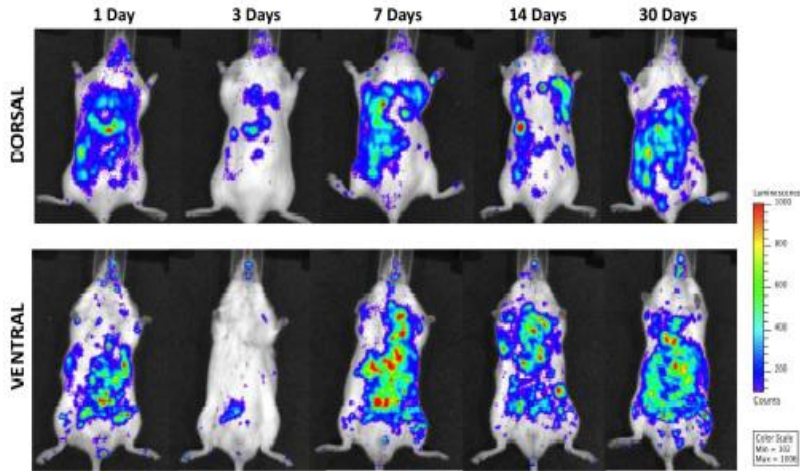
## 肝脏毒性



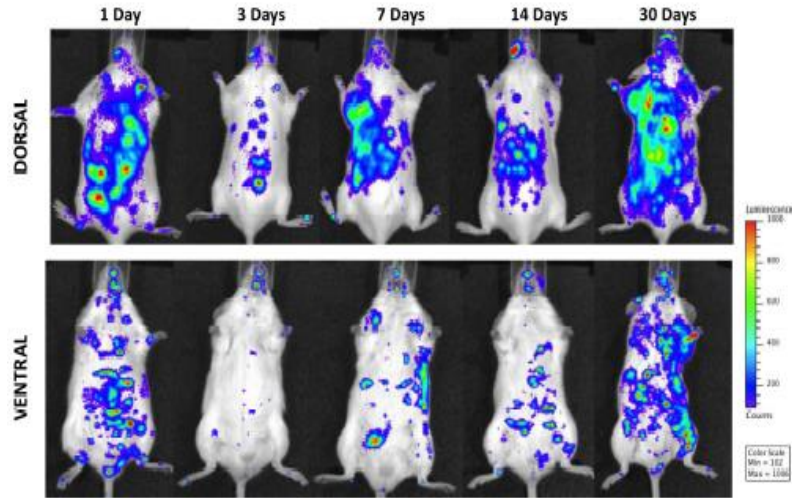


# 全身损伤模型&神经毒性

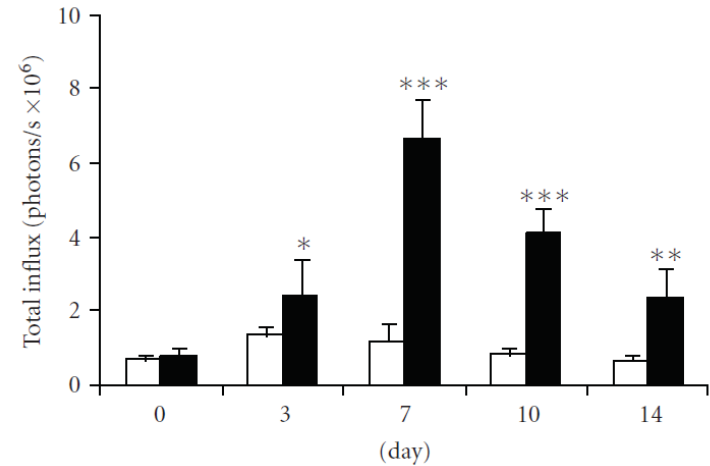
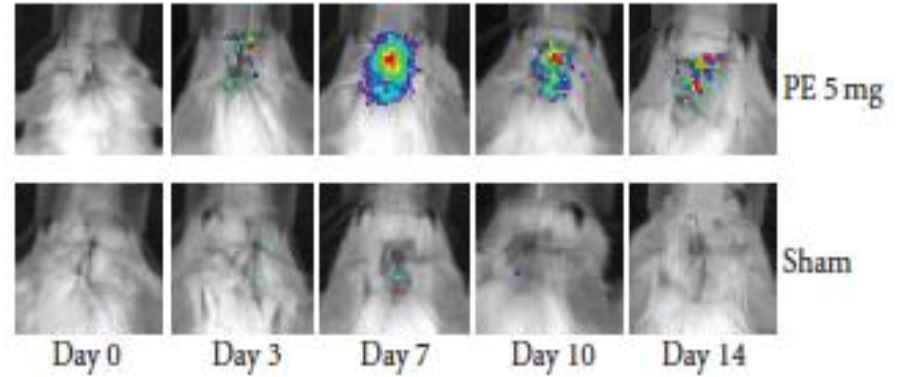
Whole-body Blast Exposure (No Protection)



Blast with Head Protection

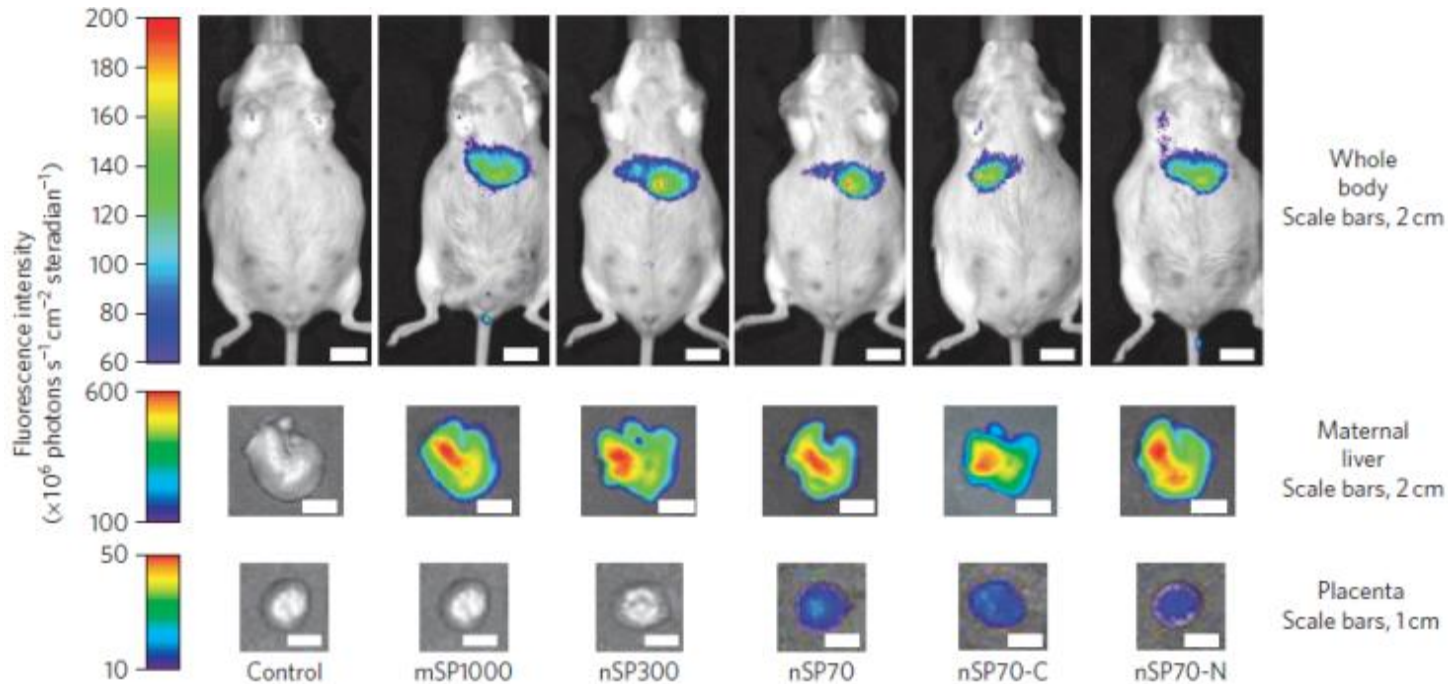


爆照损伤后，检测MPO酶活性



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。

# 纳米颗粒生殖毒性评价



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

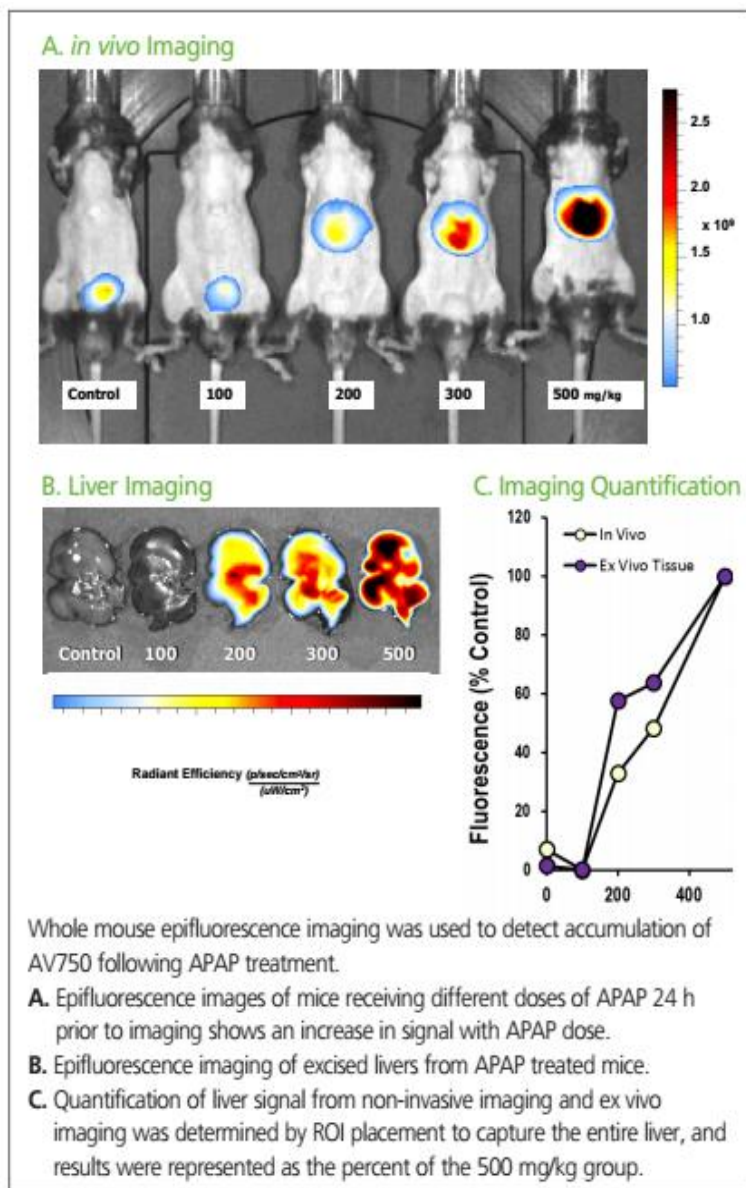
Scale bar, 1cm



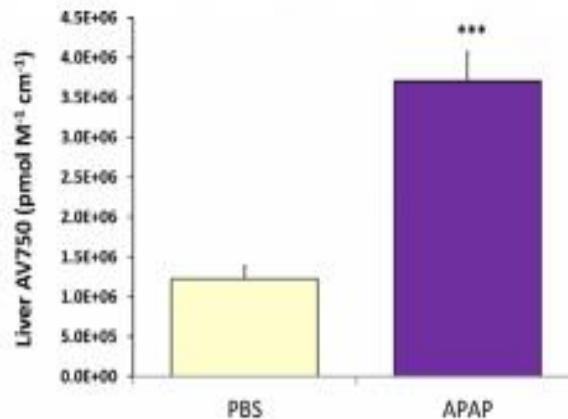
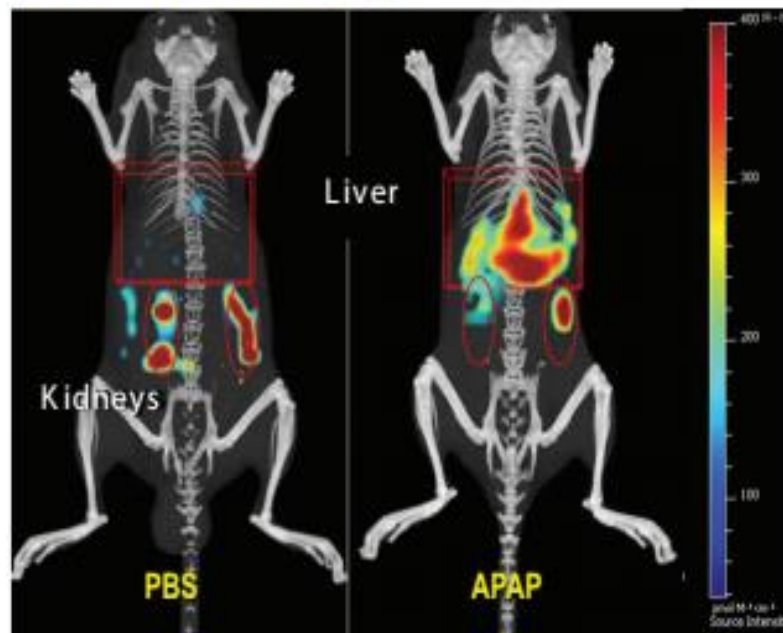
Cont mSP1000 nSP300 nSP70 TiO<sub>2</sub> Fullerene C<sub>60</sub> nSP70-C nSP70-N

nSP70和TiO<sub>2</sub>产生明显毒副作用，导致胎鼠体重明显偏轻；  
较大粒径nSPs、COOH/NH<sub>2</sub>表面修饰的nSPs、Fullerene均无明显胎毒性

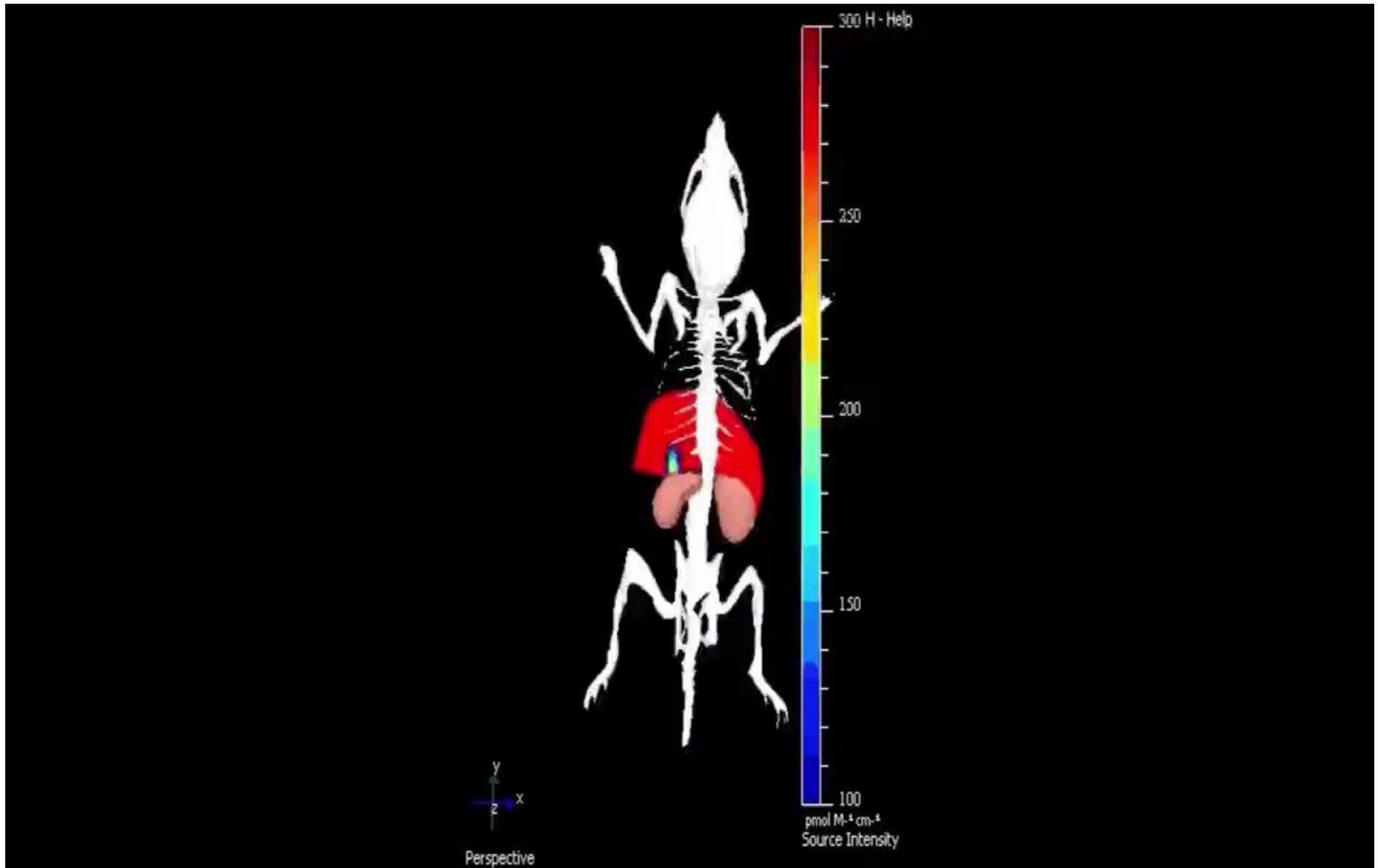
# 肝脏毒性： 肝脏细胞凋亡



## A. 3D IVIS FLIT/CT Imaging



# 肝脏毒性：肝脏纤维化

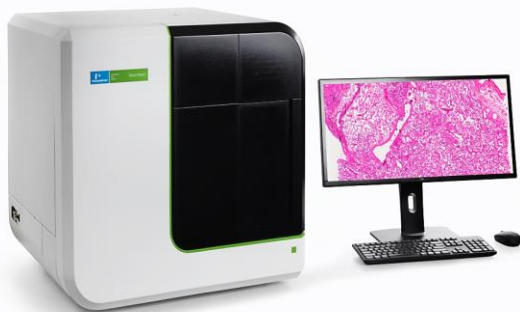




# 全自动定量病理分析系统Vectra Polaris



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

5

## Breast Cancer

HER2 (rabbit)-Red

ER (rabbit)-Green

PR (mouse)-Yellow

Ki-67 (mouse)-Purple

Nuclei (DAPI)-Blue

PLEX



同源多标 准确定量

7

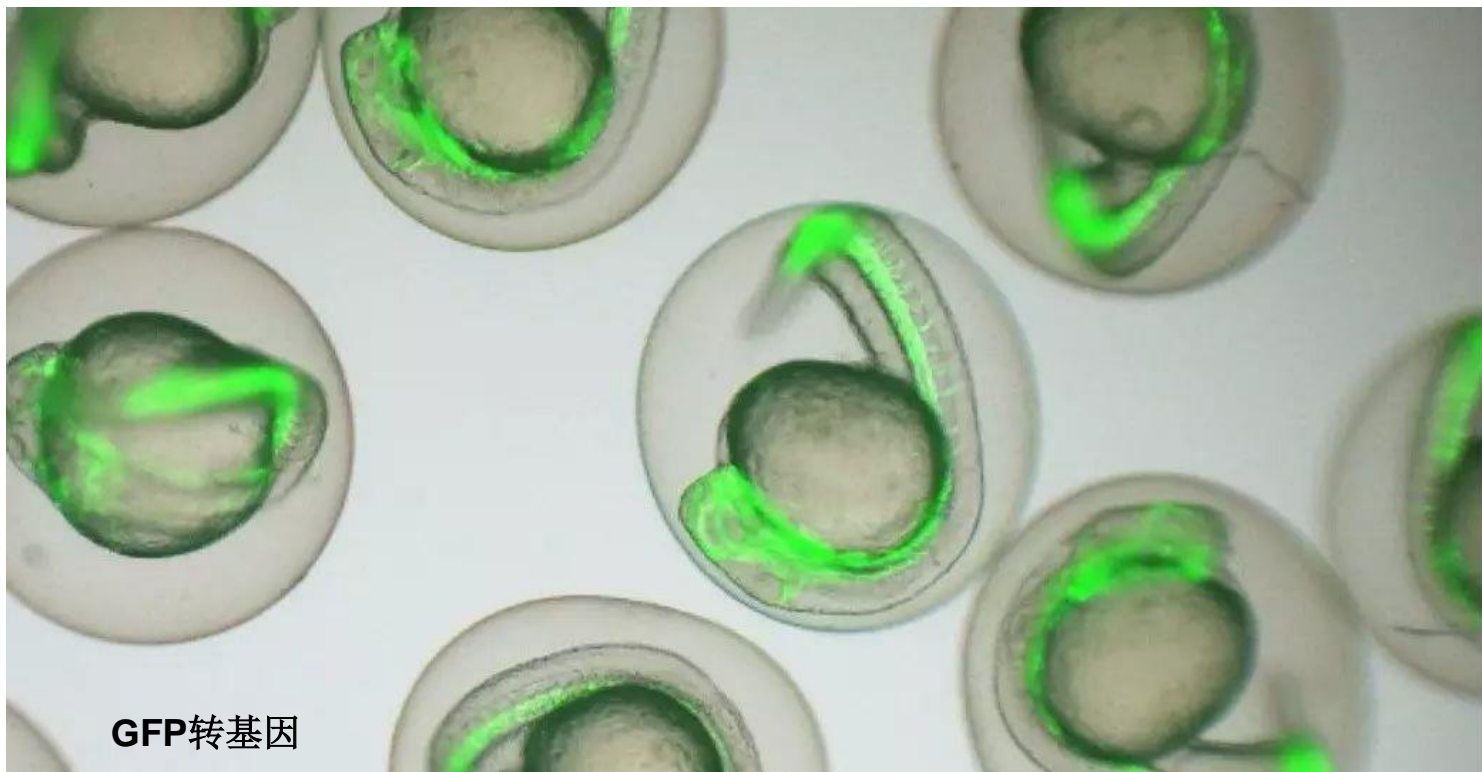
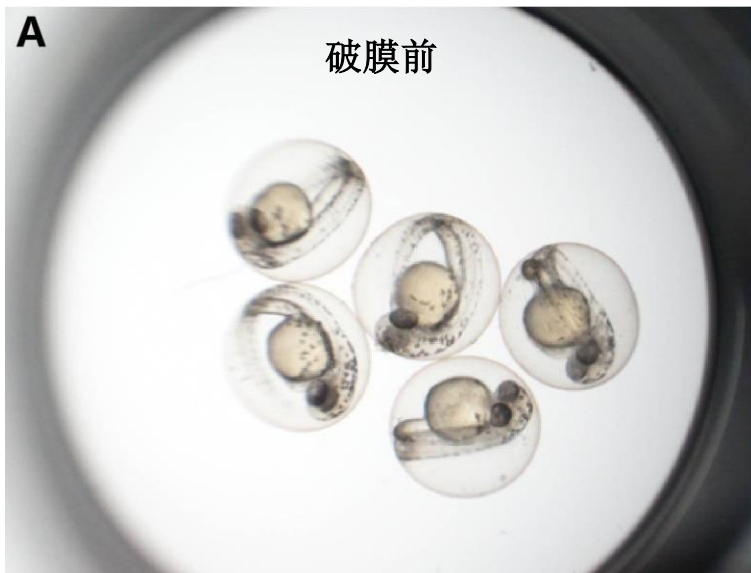
Breast Cancer  
CD4(mouse)-Green  
CD8(mouse)-Red  
CD20(mouse)-Orange  
FoxP3-(rabbit)-Purple  
PD-L1(rabbit)-Yellow  
CK(mouse)-Cyan  
Nuclei(DAPI)-Blue

PLEX



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



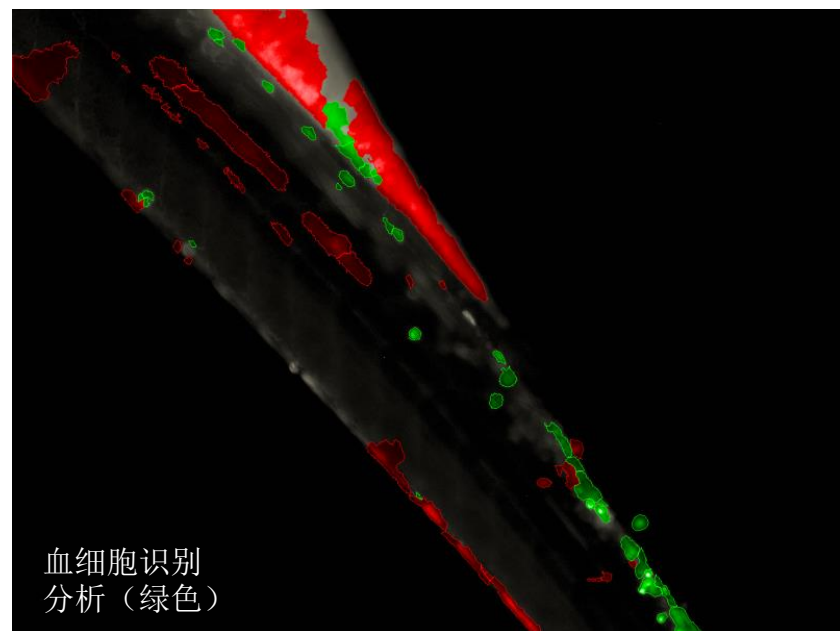
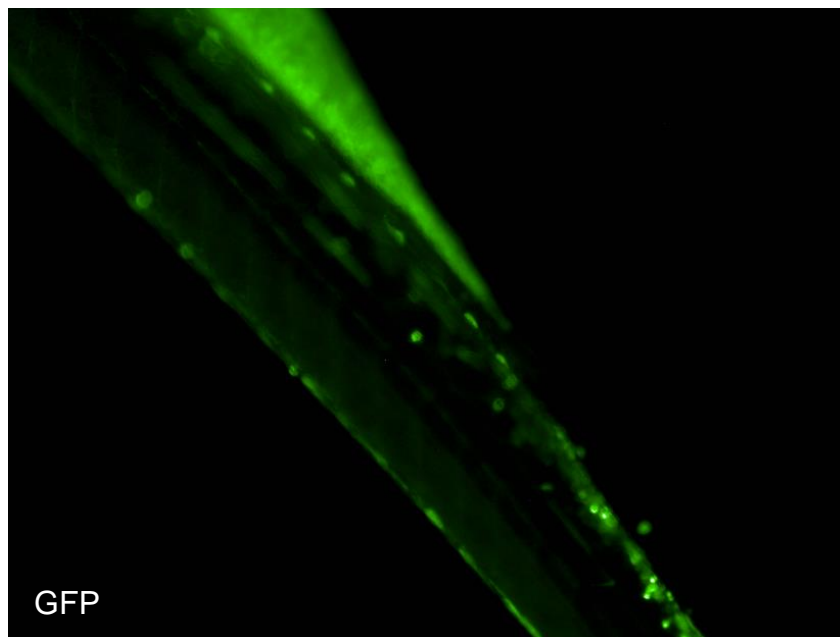
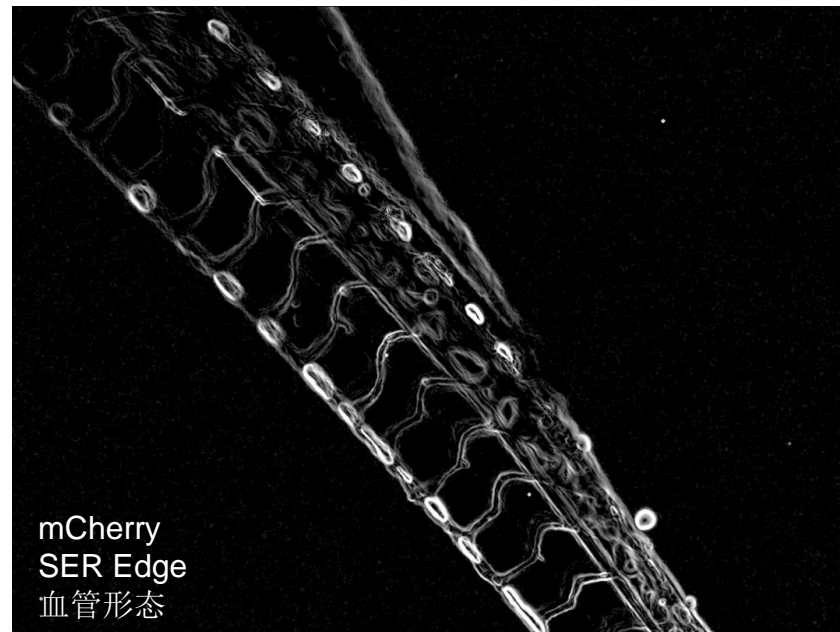
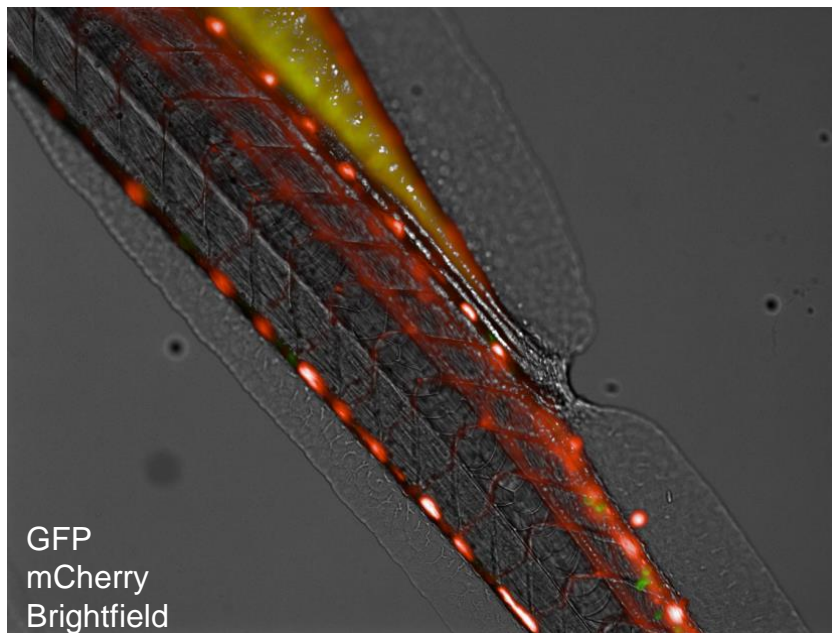
图片来自：上海南方模式动物中心

环境检测与化学品风险评估

斑马鱼：养在96孔板里的动物模型



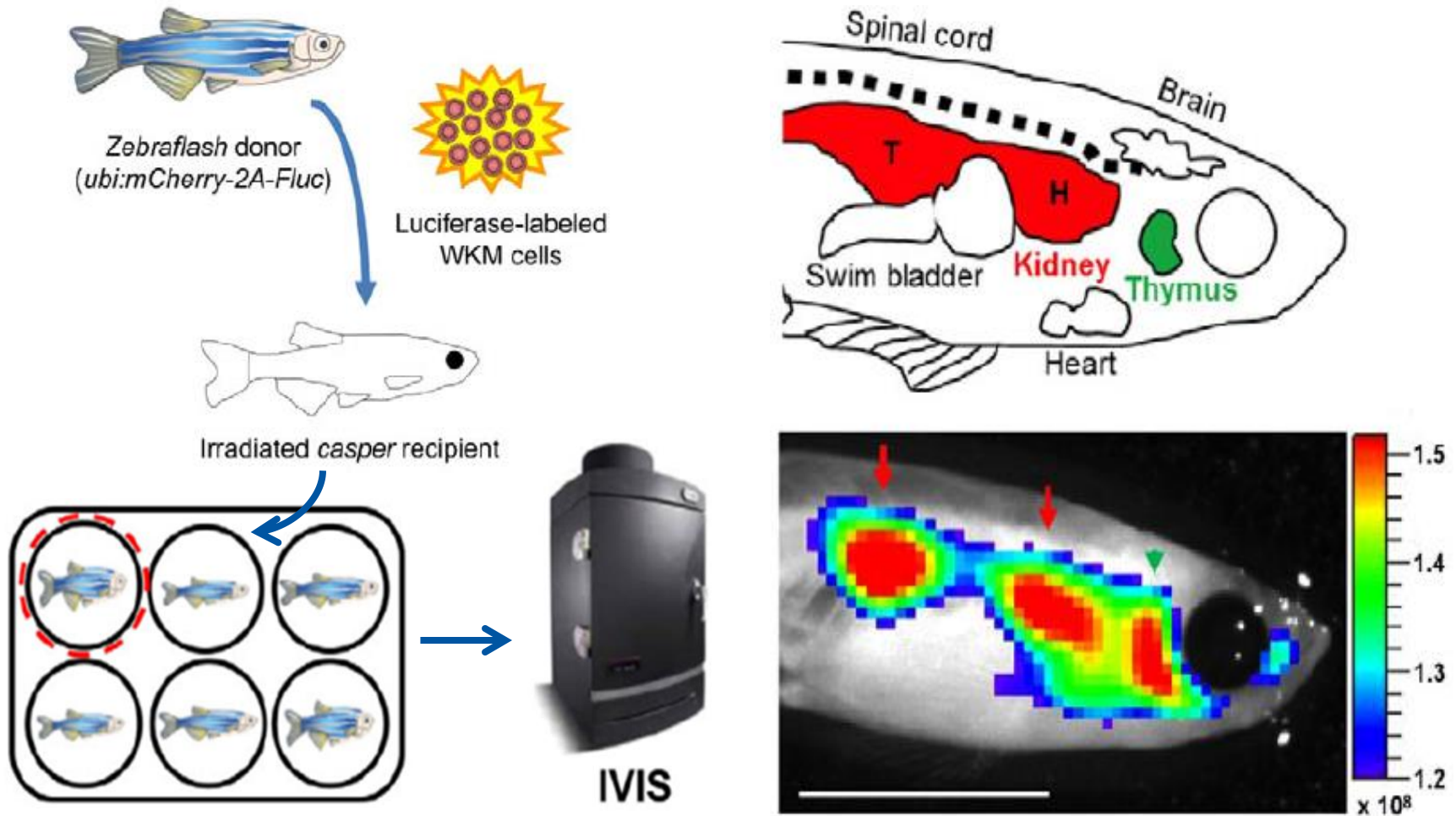
# 高内涵显微成像分析斑马鱼血管和血细胞



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

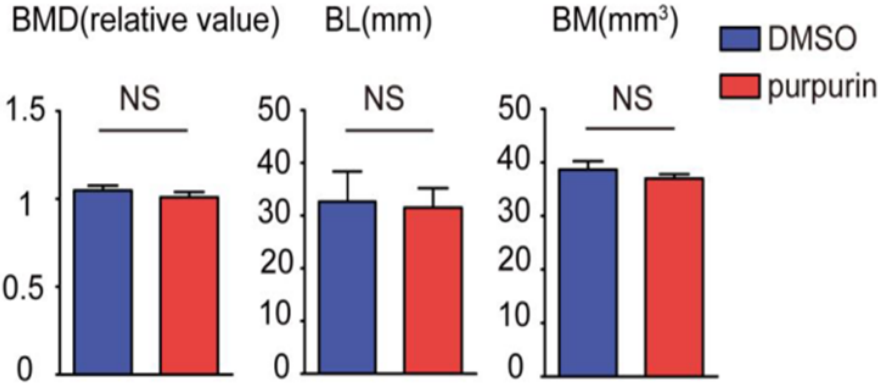
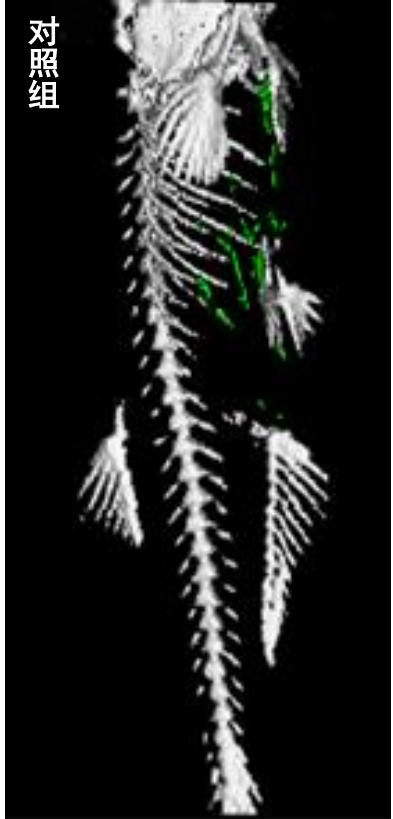
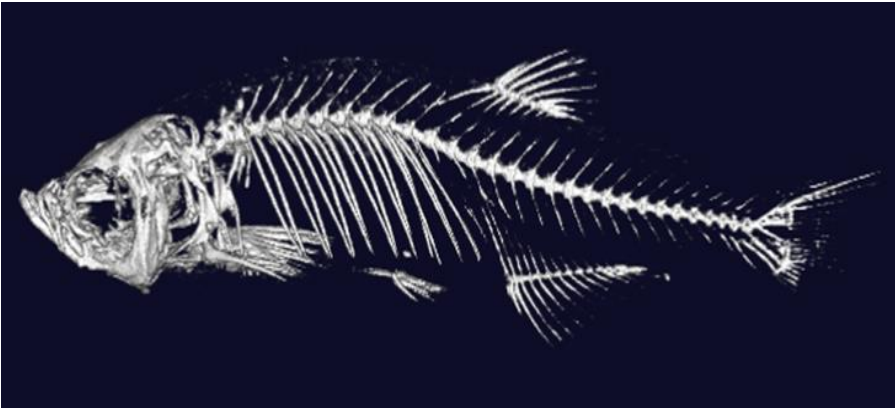
Chen-Hui Chen<sup>1</sup>, Ellen Durand<sup>2</sup>, Jinhu Wang<sup>1</sup>, Leonard I. Zon<sup>2</sup> and Kenneth D. Poss<sup>1,\*</sup>



# 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 — Discovery Solutions





## Thank you! Any Questions?

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