

# IVIS<sup>®</sup>小动物活体 光学成像系统

杜菡影

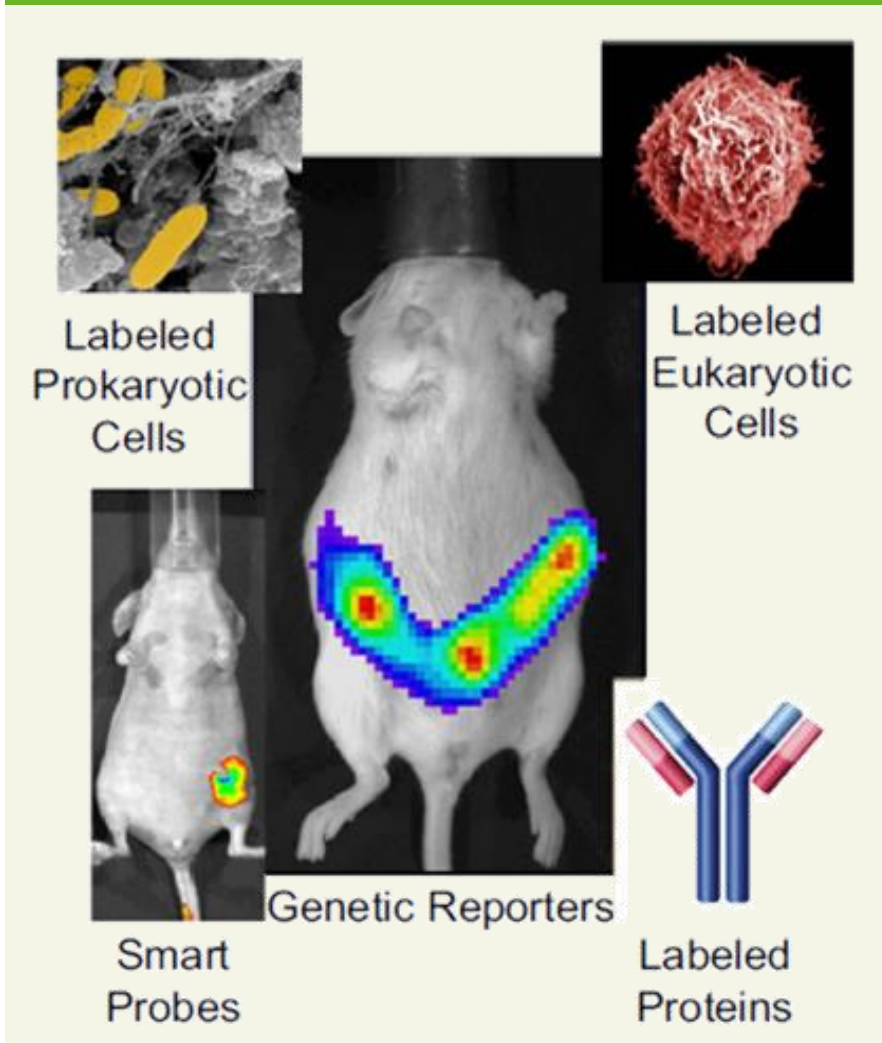
Hanying.du@perkinelmer.com

PerkinElmer活体影像INV团队



**小动物活体光学成像**——是通过一定的方式对研究对象进行**光学标记**，使其具有发光的性质，再通过**成像技术及设备**对光信号进行采集成像。

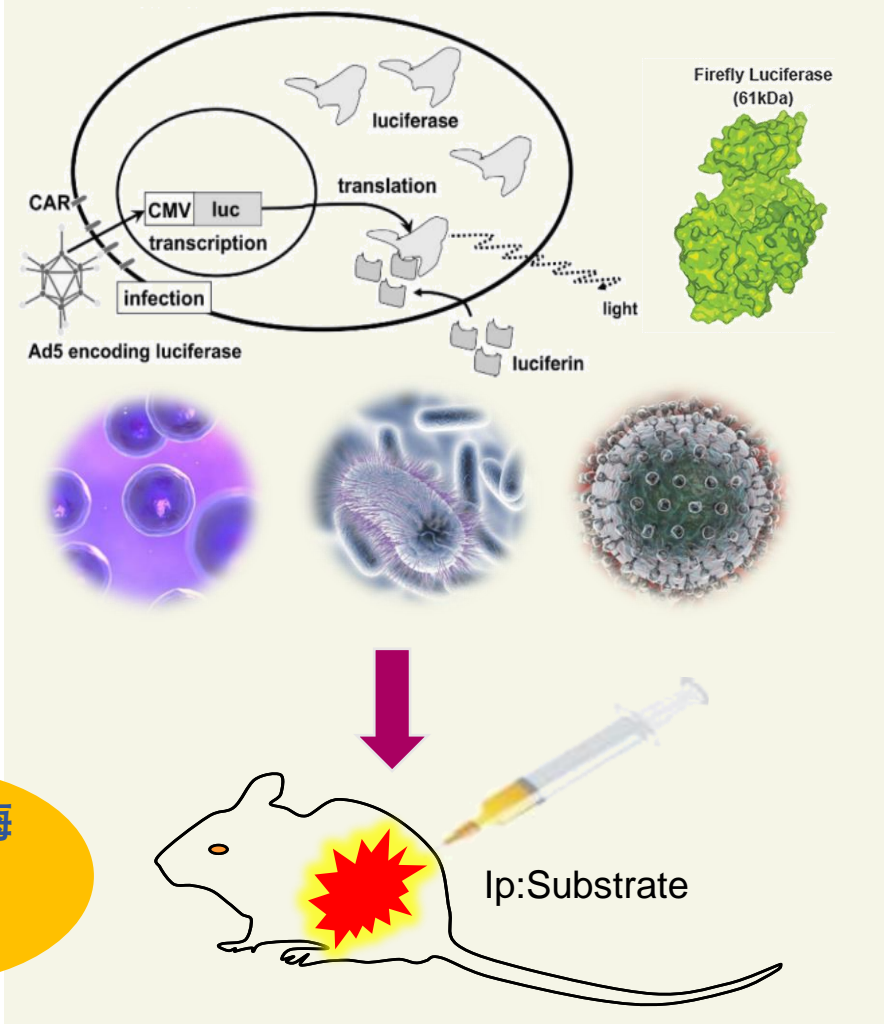
## 光学标记



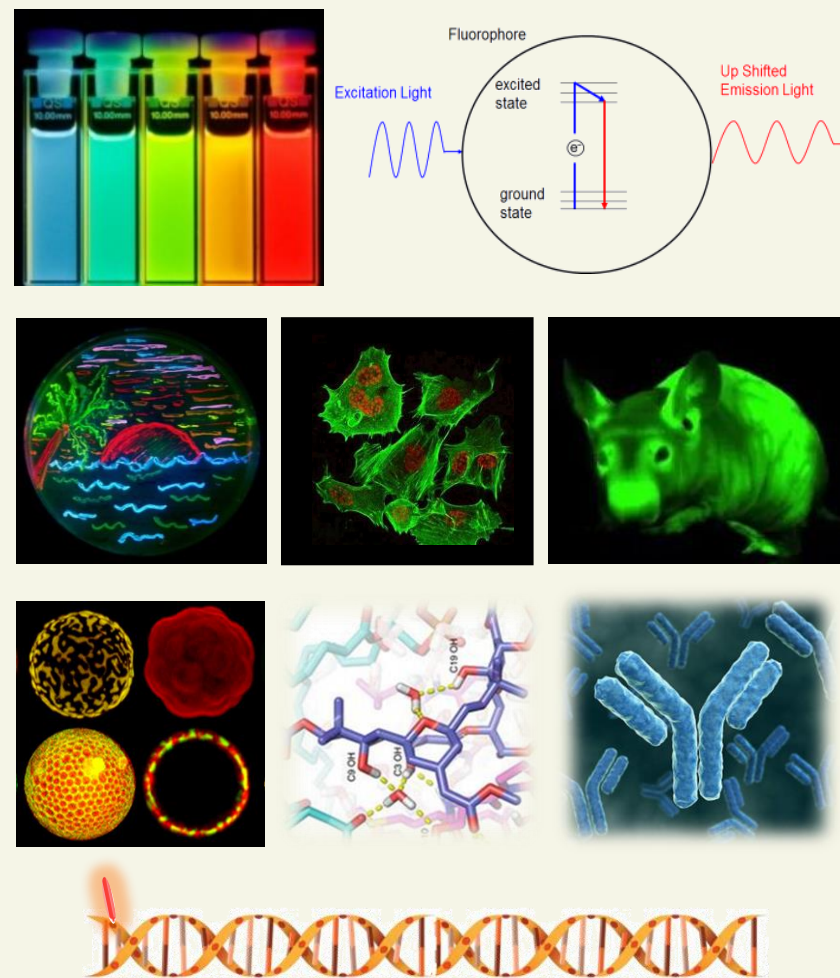
## 成像技术及设备



## Bioluminescence



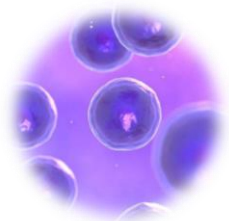
## Fluorescence



# 标记方式-小结

## 检测对象

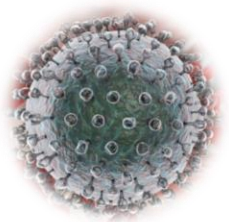
## 标记方式



荧光素酶 (长期)  
荧光蛋白 (长期)  
脂溶性荧光染料 (短期)  
荧光探针



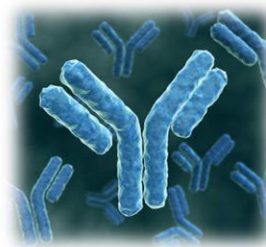
荧光素酶 (长期)  
荧光蛋白 (长期)  
荧光探针



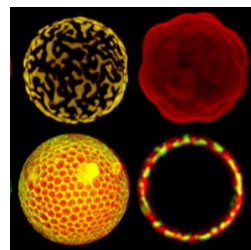
荧光素酶 (Nluc/Gluc)

## 检测对象

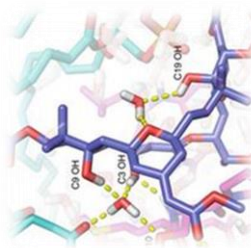
## 标记方式



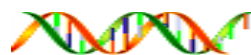
活化荧光染料-共价结合



荧光染料-包裹、包埋  
荧光蛋白-膜上表达  
活化荧光染料-膜上蛋白共价结合

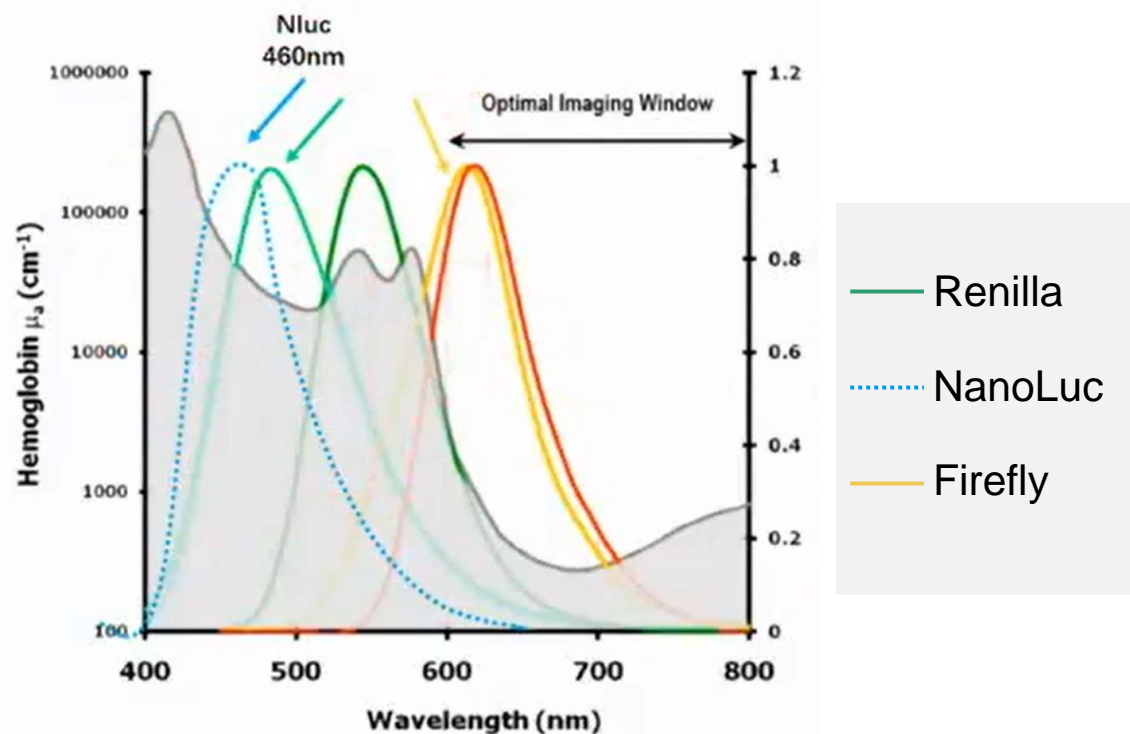


活化荧光染料-共价结合、从头合成



活化荧光染料-共价结合

# 三种常用荧光素酶差异



## ○ Firefly Luciferase 萤火虫荧光素酶

发射波长560nm，体内会红移至610nm，组织穿透性更好，适用于动物内脏等较深部位的组织成像；

## ○ Renilla Luciferase 海肾荧光素酶

发射波长480nm，产生的蓝光组织穿透能力较弱，适用于动物浅表组织成像；

## ○ NanoLuc Luciferase 海虾荧光素酶

发射波长460nm，自然界提取光型号强度较弱，但有改良版本，适用于病毒等对报告基因片段大小有限制的标记场景。

# 生物发光活体成像试验流程

## 荧光素酶:

- Firefly Luciferase
- Renilla Luciferase
- NanoLuc Luciferase

## 载体

## 稳转细胞系

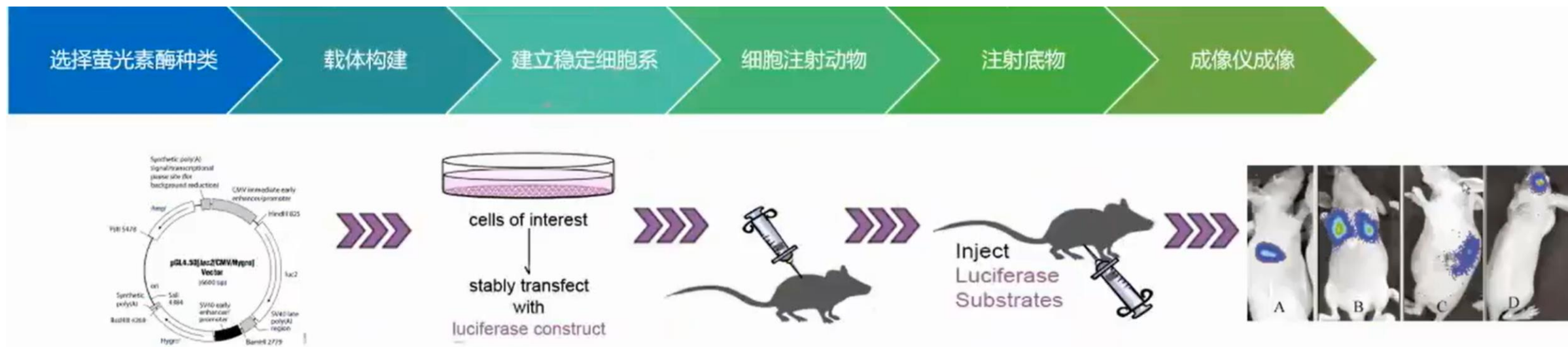
## 转基因动物

## 荧光素酶底物:

- D-Luciferin
- Coelenterazine h
- Inflammation Probe
- .....

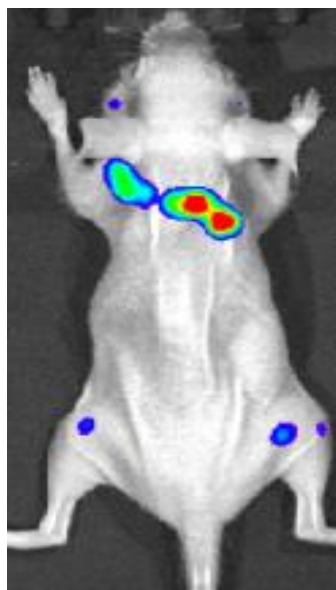
## 成像设备:

- IVIS Lumina III
- IVIS Lumina S5 & X5
- IVIS Spectrum
- IVIS Spectrum CT
- .....

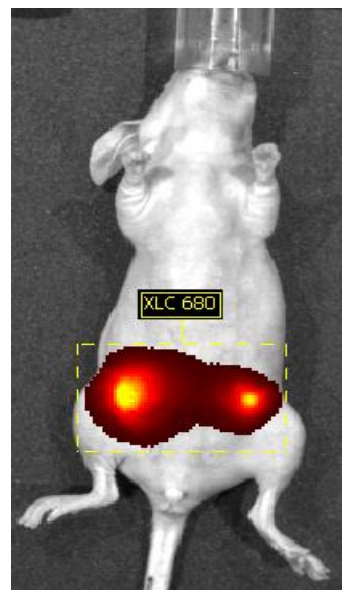


# IVIS Spectrum 成像模块

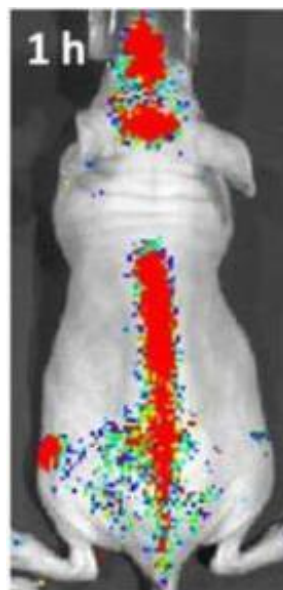
生物发光/化学发光



荧光



切伦科夫成像



多探针成像



三维荧光成像



三维生物发光成像

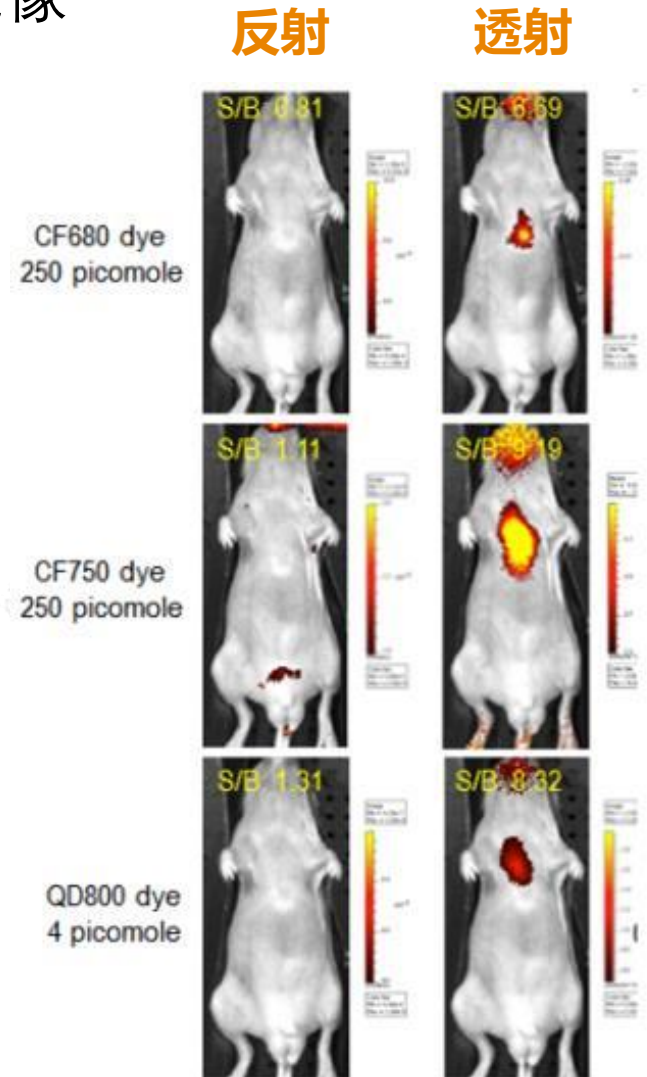
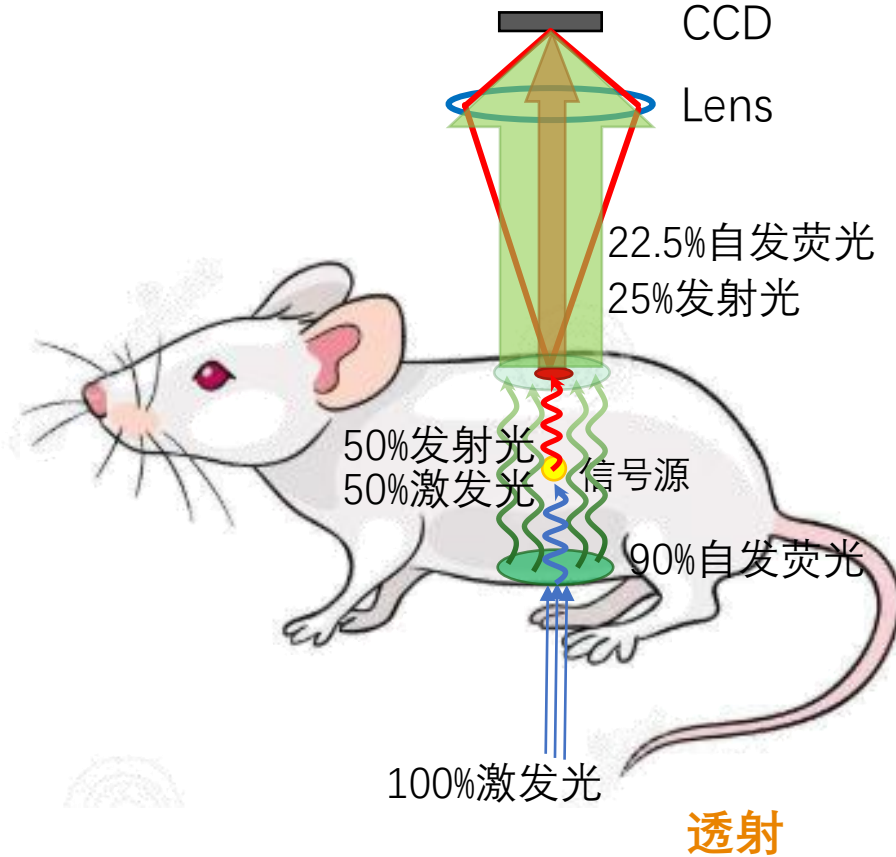
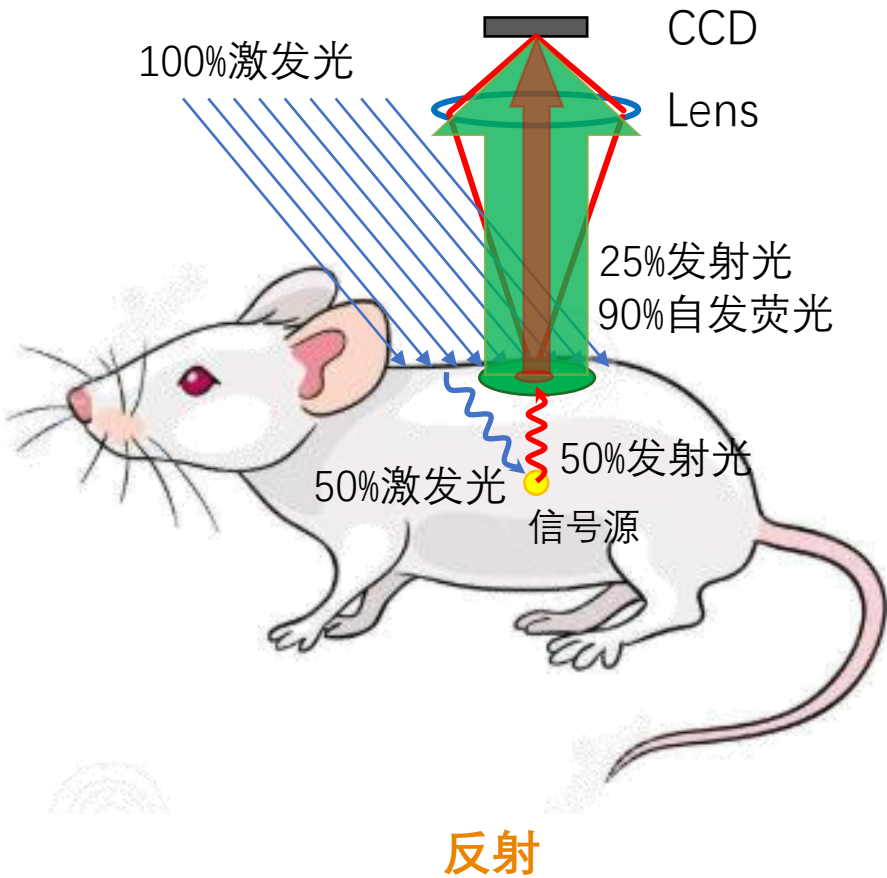


IVIS Spectrum 是业内目前成像模式最多最全的系统，满足几乎所有活体光学成像需求，同时可以与结构成像模式融合，一次成像获得更多更全面的信息！



# Spectrum成像模块——透射成像

透射成像的优势：更低的自发荧光背景 → 实现深层荧光成像



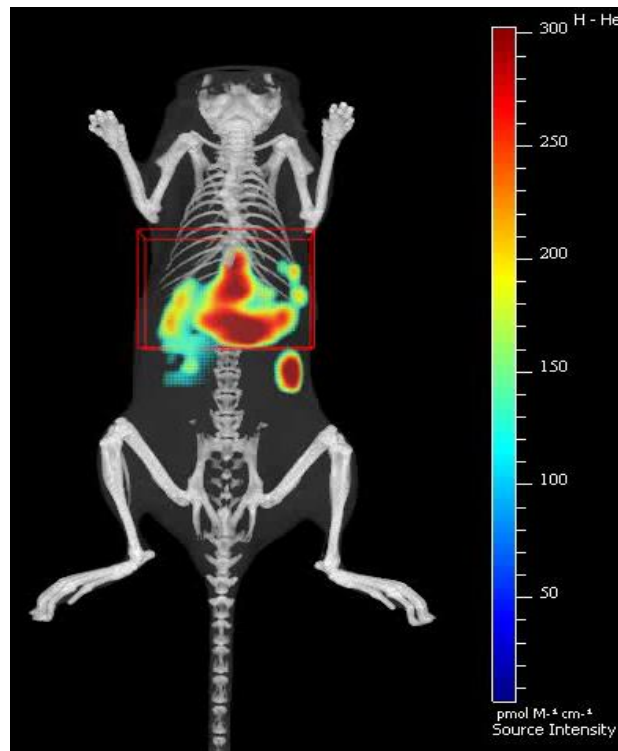
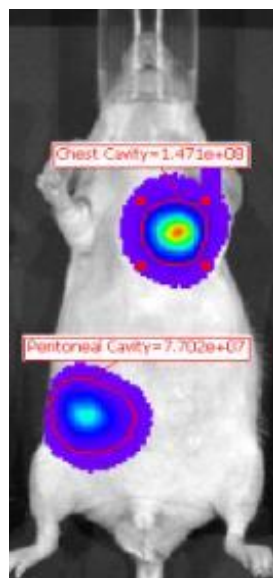
# Spectrum成像模块——活体三维成像



俯卧



仰卧

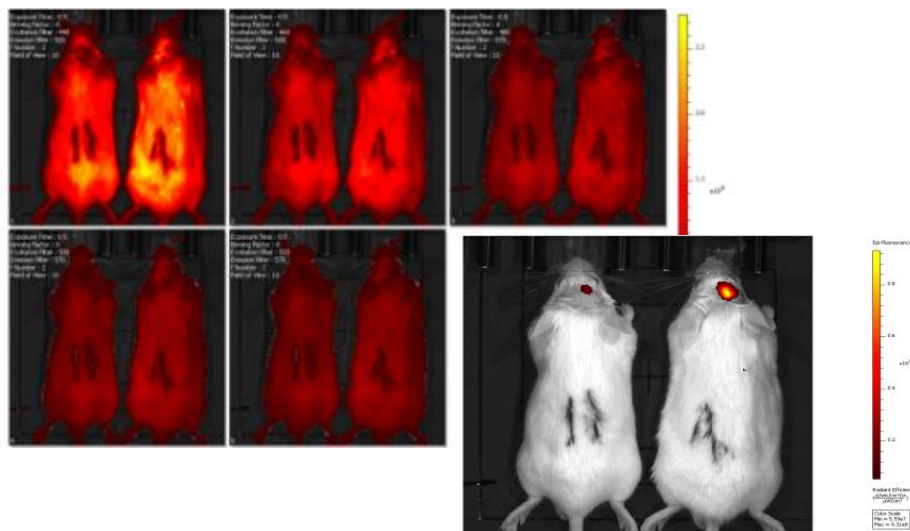
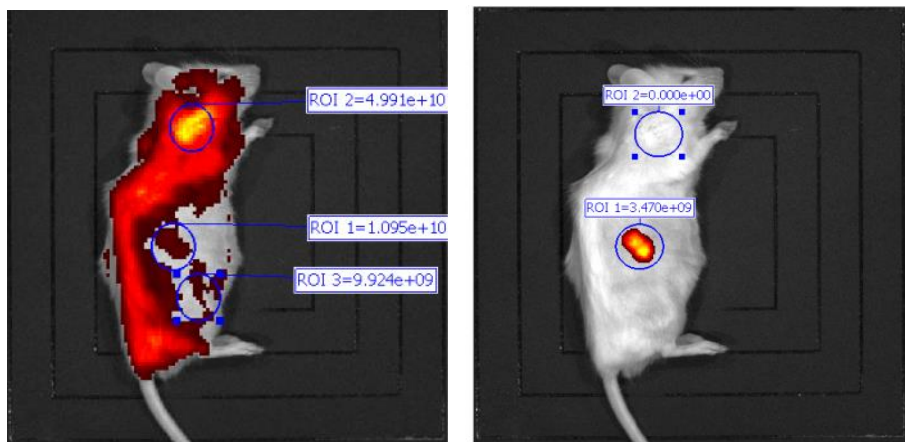


- ✓ 三维影像
- ✓ 定位信息
- ✓ 定量信息

	信号深度	信号体积	精确定量	绝对定量 (细胞数、浓度)	3D图像
2D	×	×	×	×	×
3D	√	√	√	√	√

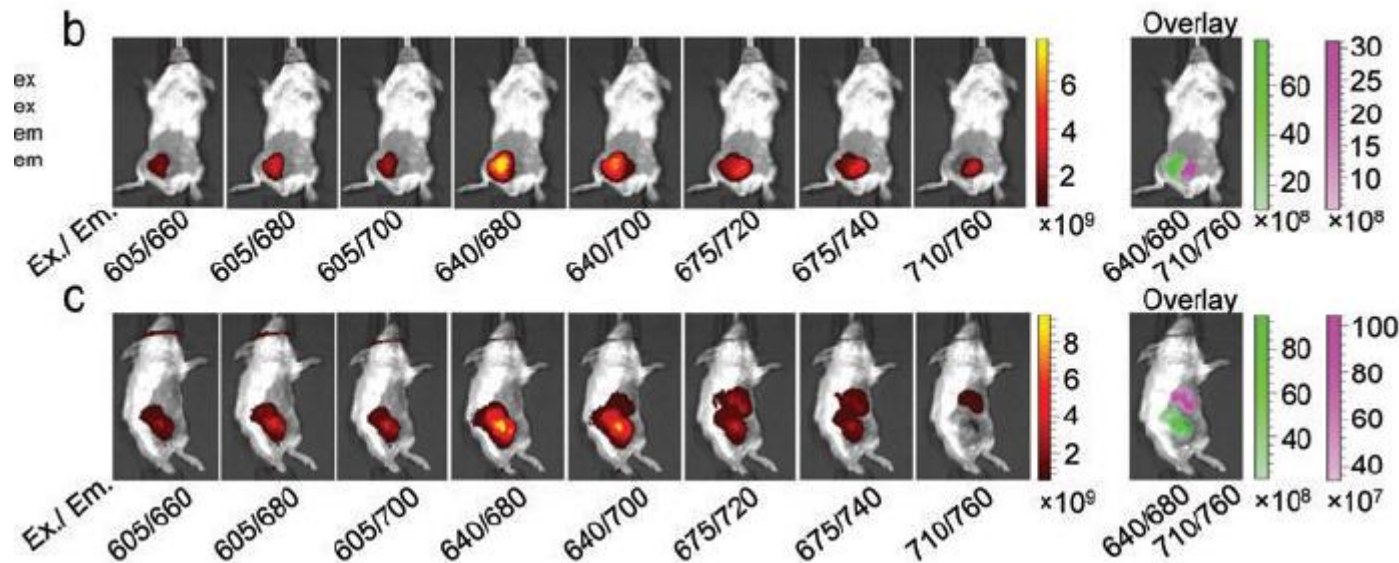
# Spectrum成像模块——光谱拆分

## 去除组织背景荧光

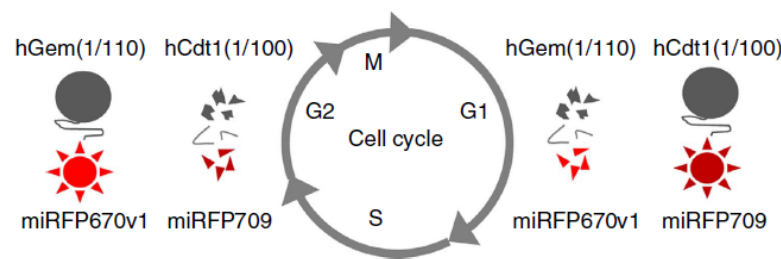


表达GFP的肿瘤细胞成像

## 多标记荧光的拆分

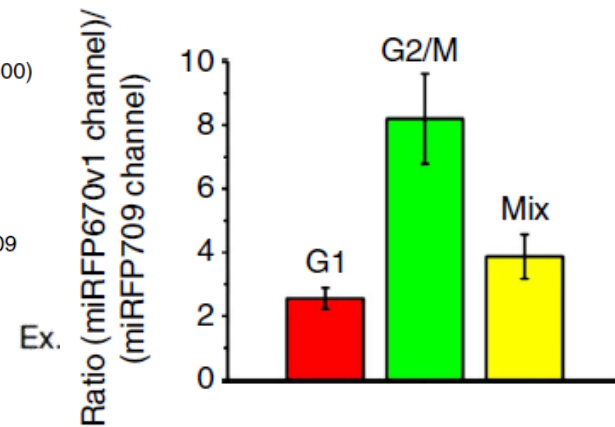


Shcherbakova and Verkhusha et al., *Nat Methods*.2014



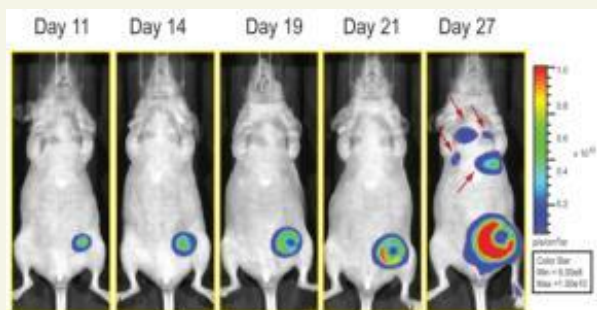
## 活体FUCCI监测细胞周期

NATURE COMMUNICATIONS, 2016 7:12405



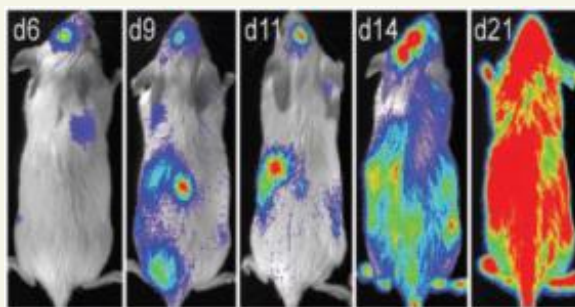


## 监测疾病的发生发展及治疗



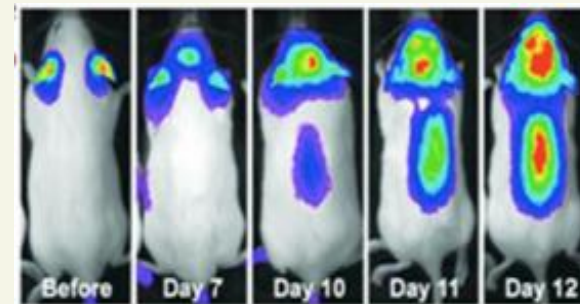
- 癌症
- 免疫性疾病
- 感染性疾病
- 代谢性疾病
- 心血管疾病
- 神经疾病

## 监测细胞动态变化



- 干细胞
- 免疫细胞
- 存活
- 分布
- 迁移
- 靶向

## 探究疾病分子机制

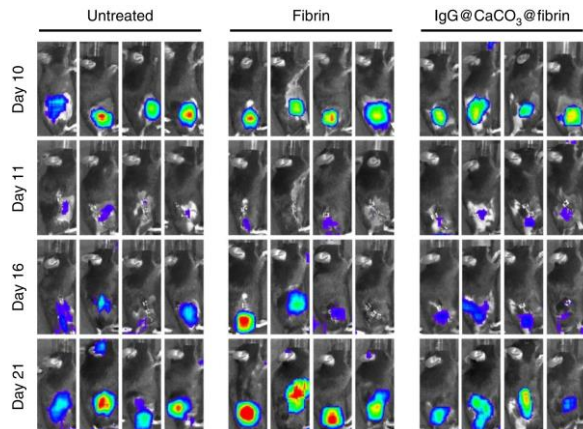


- 基因与疾病关系
- 疾病信号通路
- 细胞因子作用

# 肿瘤发生发展——肿瘤研究模型的构建

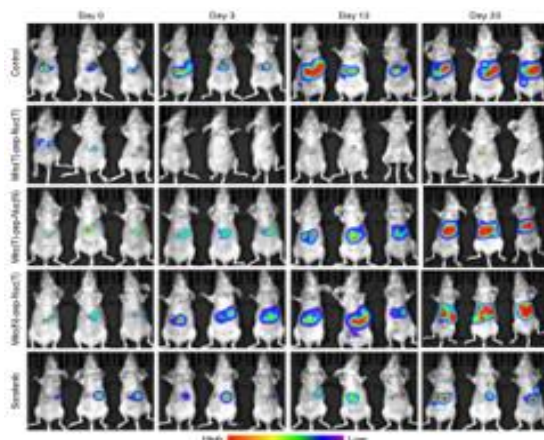
原位瘤模型

## 皮下黑色素瘤



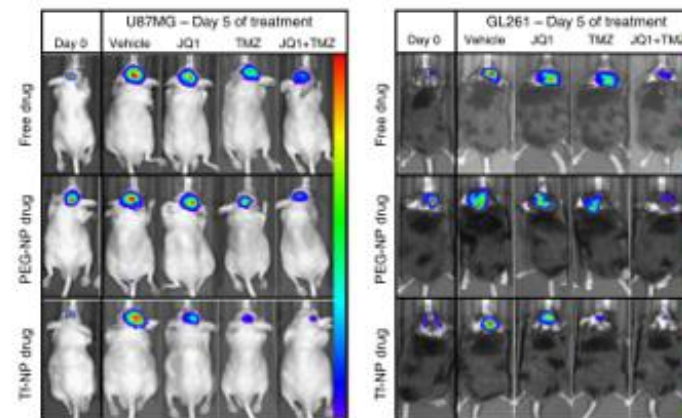
Nat. Nanotech. 2019,14: 89

## 原位肝肿瘤



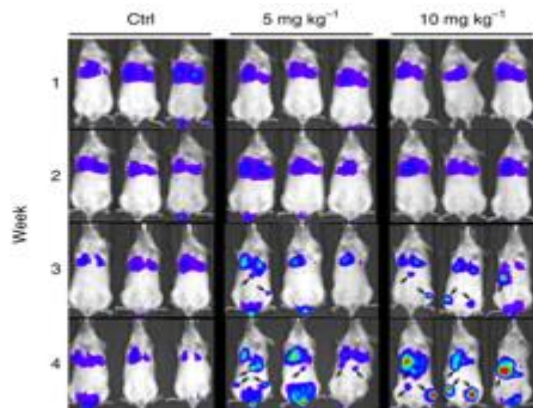
Nat. Commu. 2019,10: 3051

## 脑胶质瘤



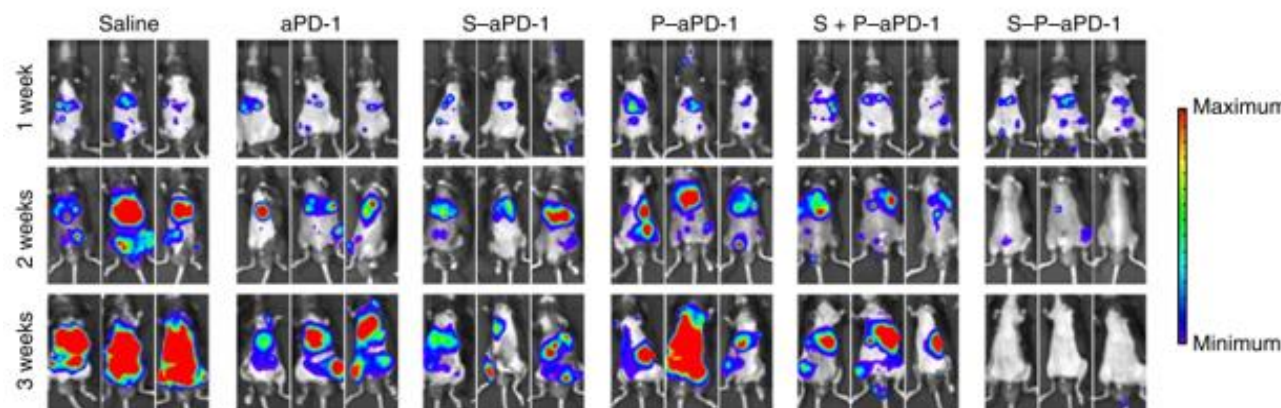
Nat. Commu. 2018,9: 1991

## 肺部转移



Nat. Nanotech. 2019,14: 279

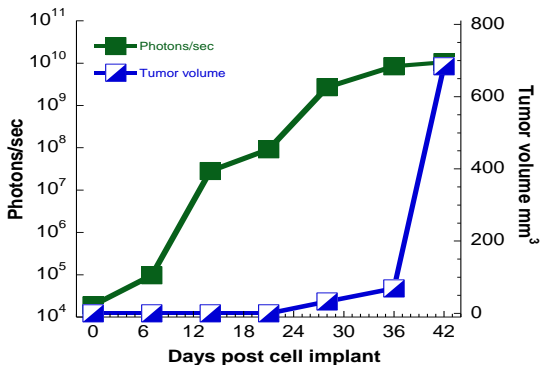
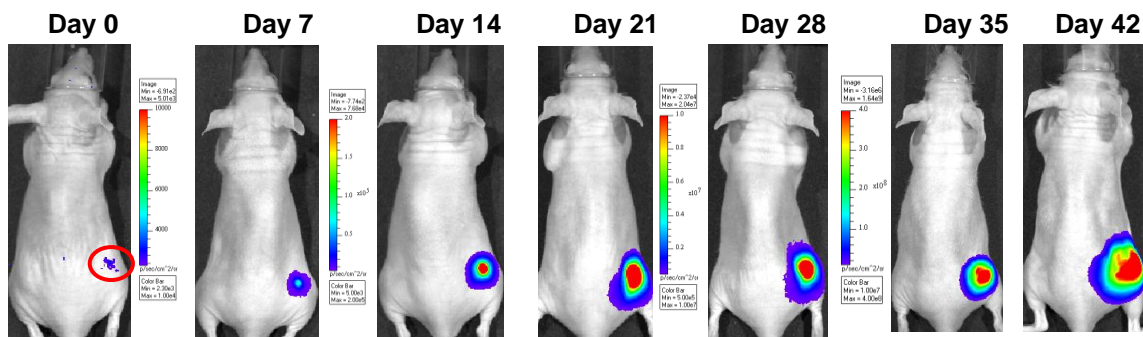
## 急性骨髓白血病



Nat. Biomed. Eng. 2019,14: 279

转移瘤模型

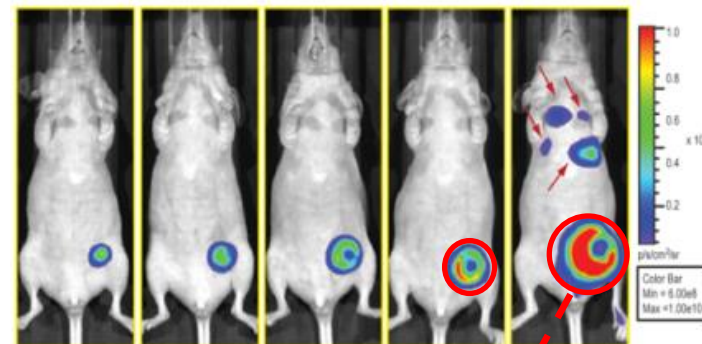
## 合理评价肿瘤的生长状态——“看的早”



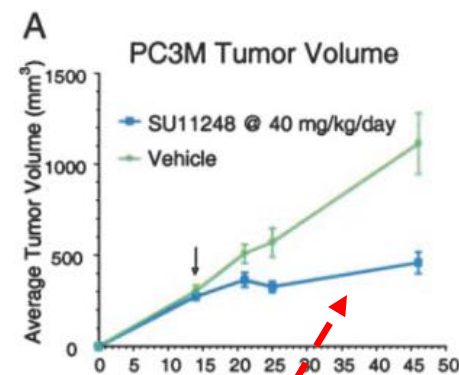
4T1-luc2

With Bioware Ultra one can start collecting data from **Day 0**, while with caliper measurements one has to wait **at least 28 days** to see any tumor growth

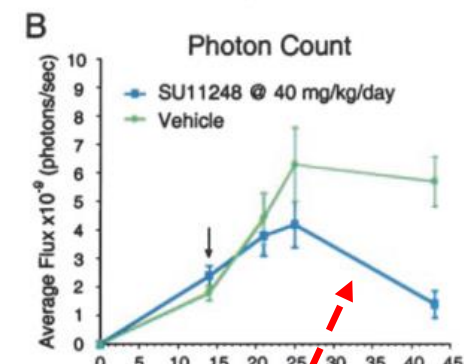
## 合理评价肿瘤的生长状态——“看的准”



瘤体虽然在变大，但实际内部已经凋亡坏死



Physical measurement  
(tumor still getting bigger)

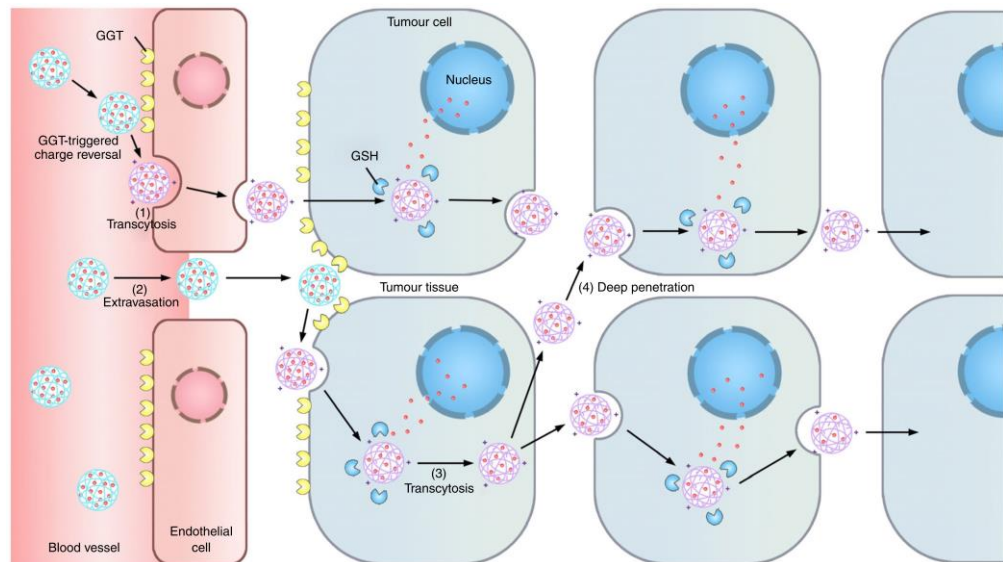


Bio-photonic imaging  
(tumor cells being killed)

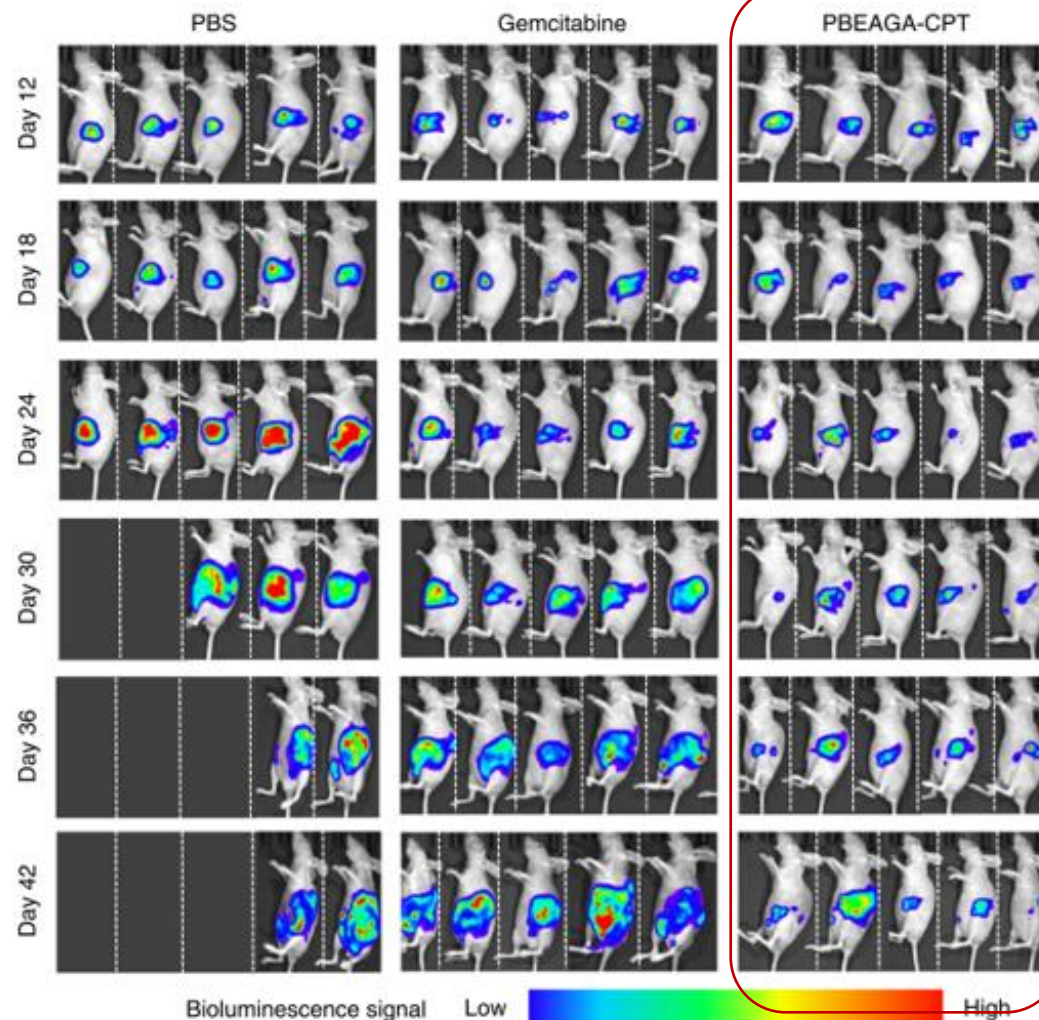
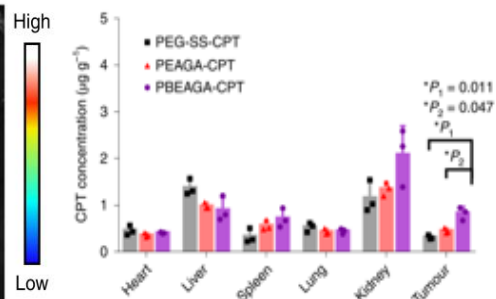
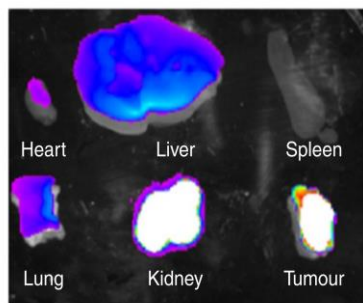
Mendel et al 2003

## 纳米药物在肿瘤内的主动渗透

### 肿瘤靶向纳米药物的体内输送过程 (CAPIR)



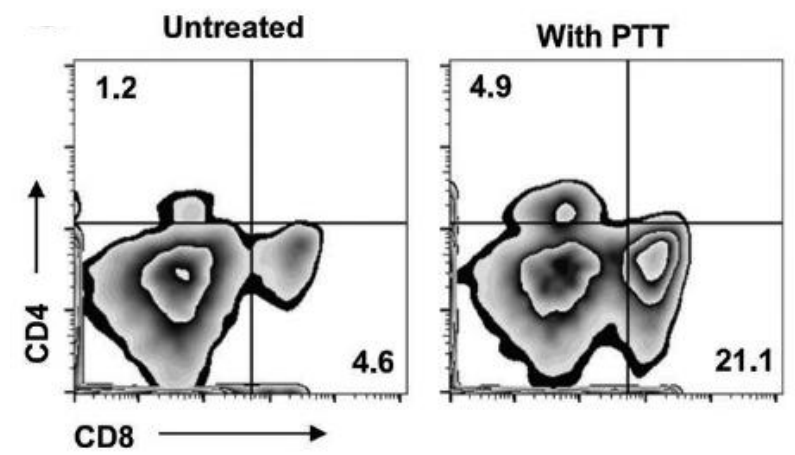
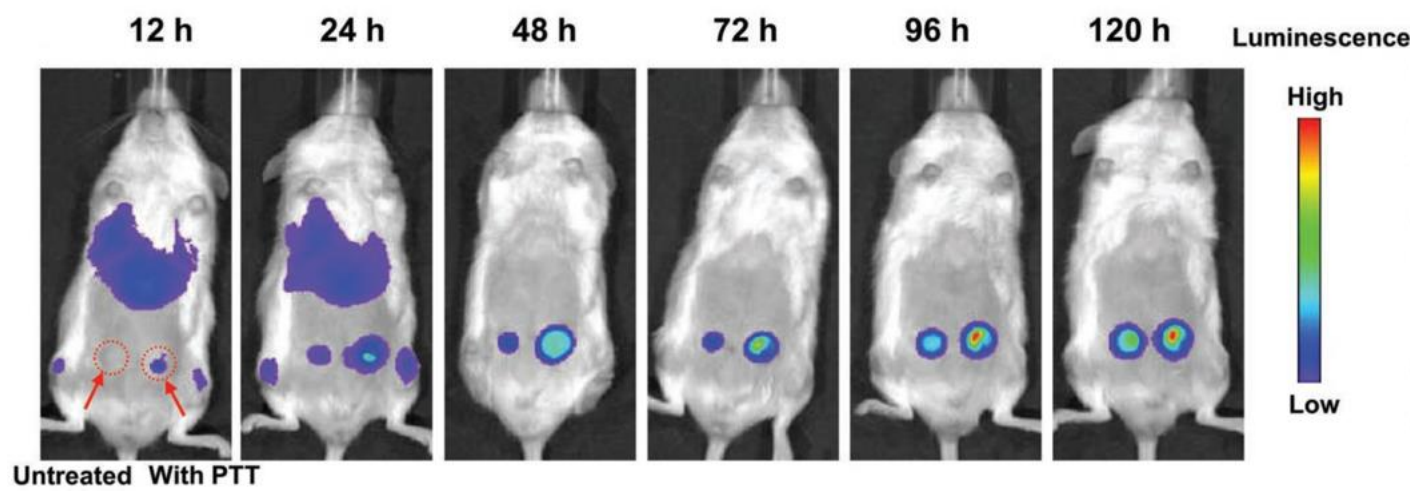
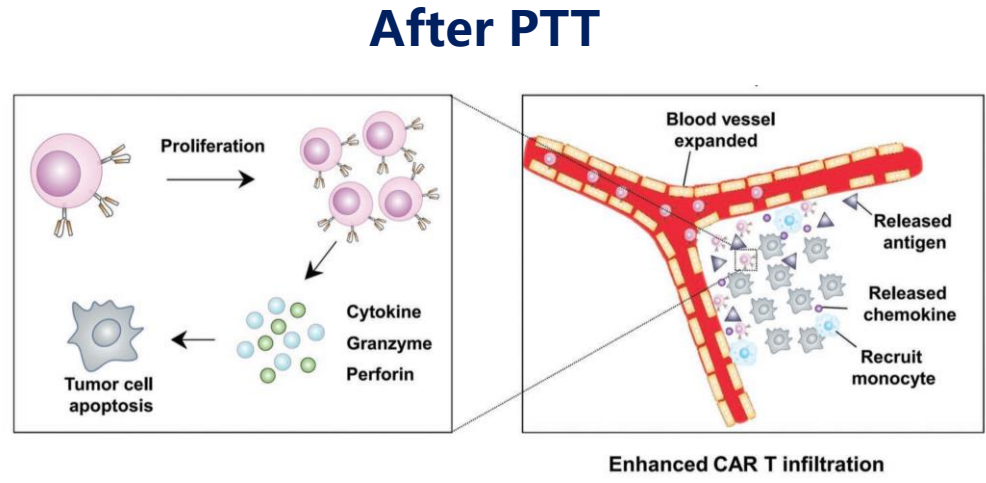
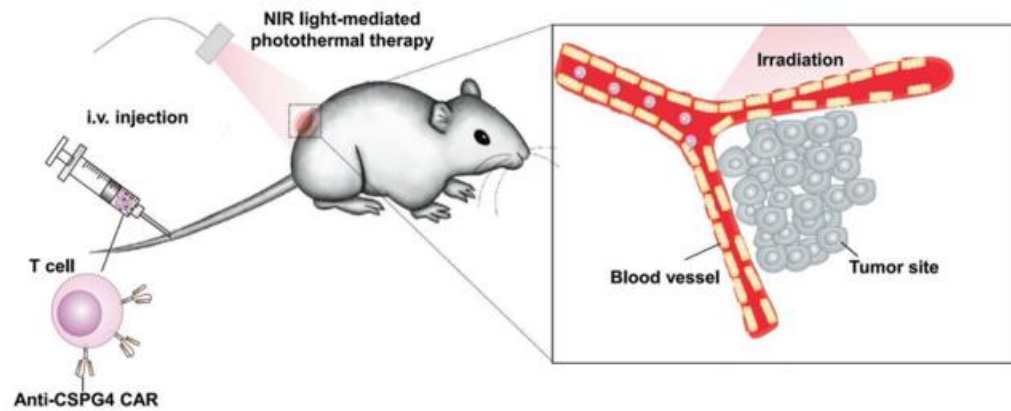
靶向肿瘤





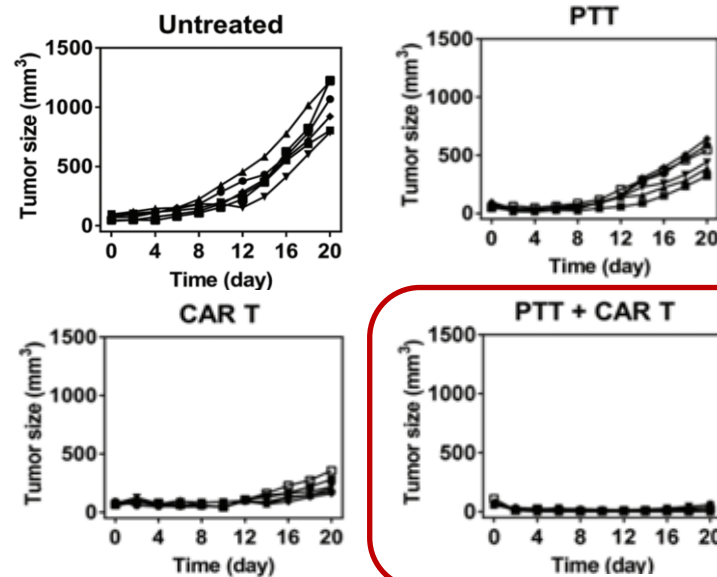
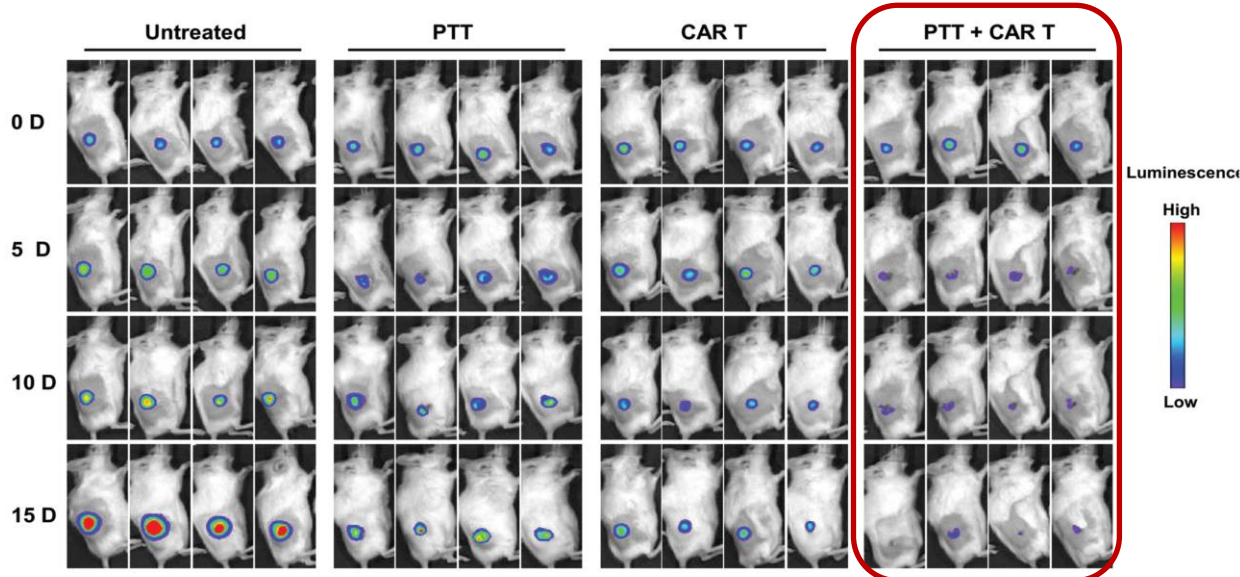
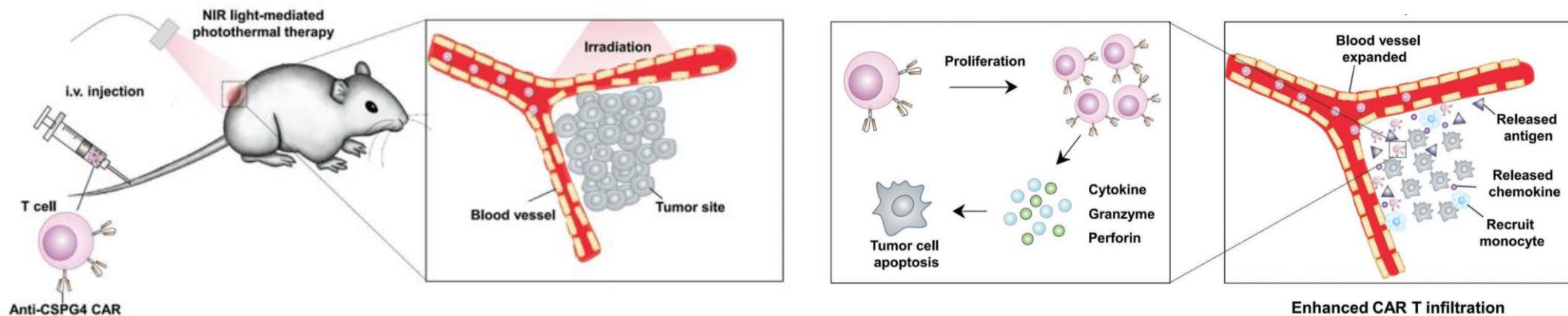
# 肿瘤治疗---CAR-T治疗

## CAR-T细胞靶向肿瘤



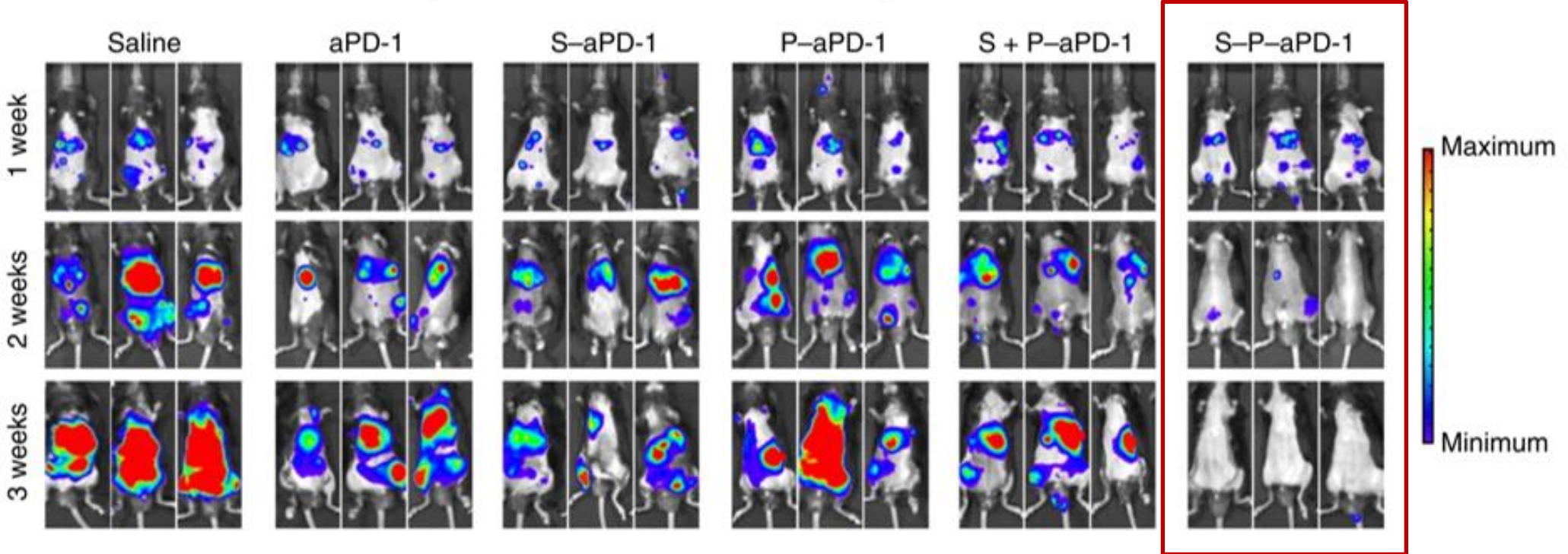
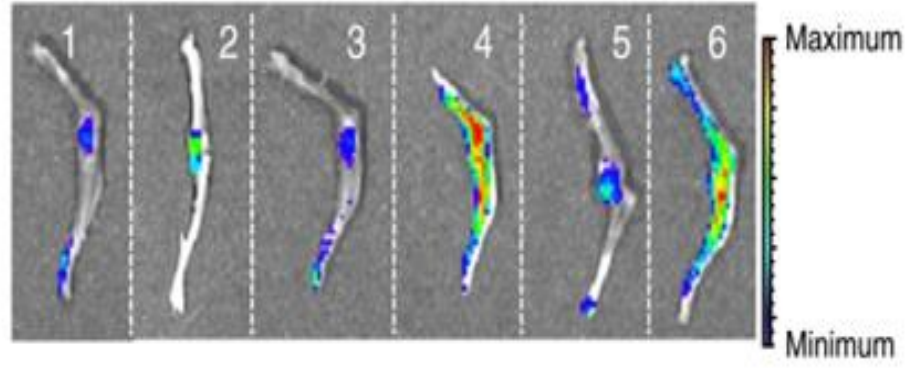
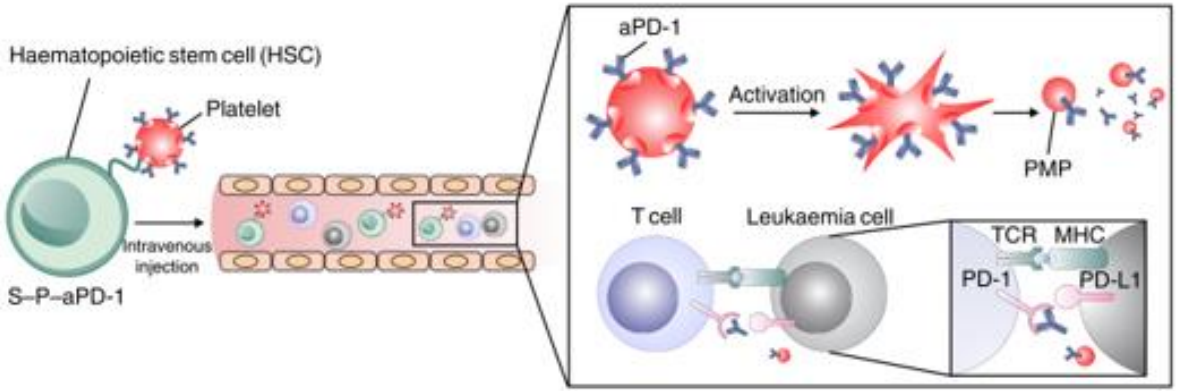
## CAR-T 细胞结合光热作用 (PTT) 治疗实体肿瘤

After PTT



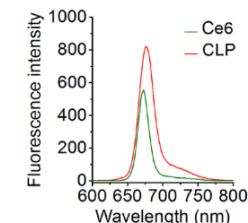
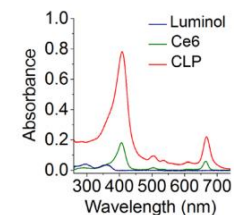
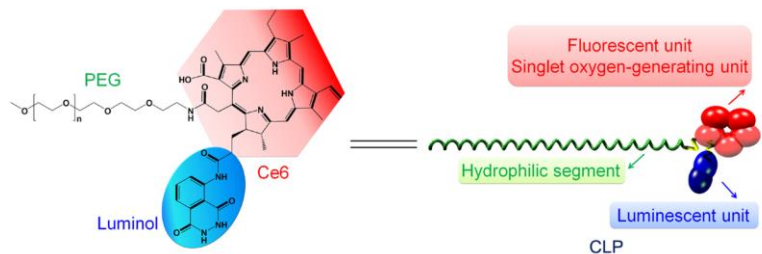
# 肿瘤治疗——干细胞治疗

HUMAN HEALTH | ENVIREMENTAL HEALTH



# 炎症的发生

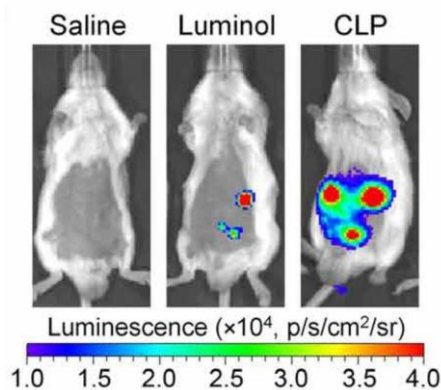
HUMAN HEALTH | ENVIRONMENTAL HEALTH



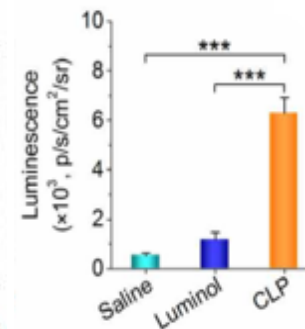
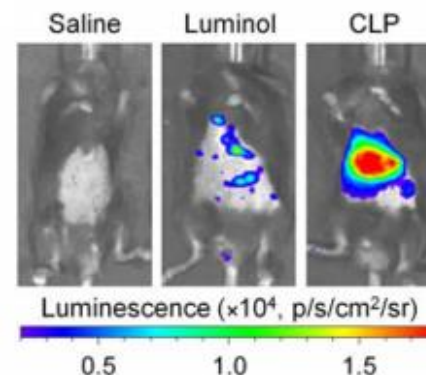
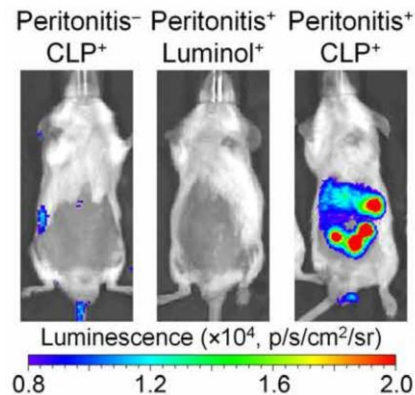
## 腹膜炎模型

## 急性肝损伤模型

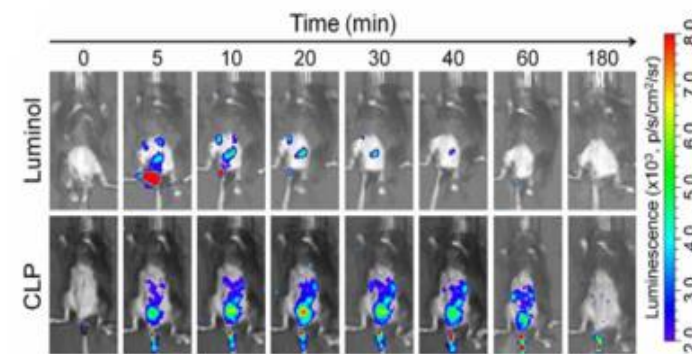
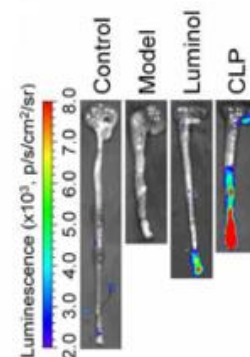
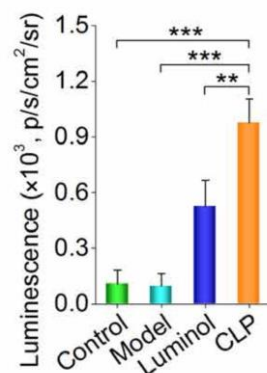
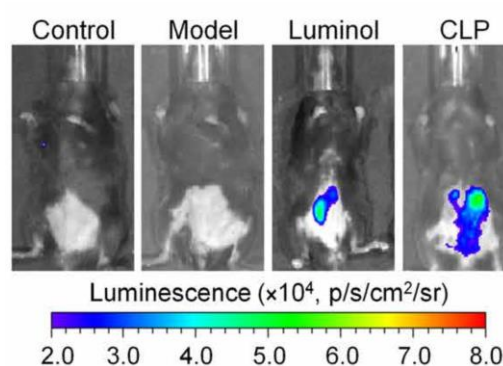
腹腔注射



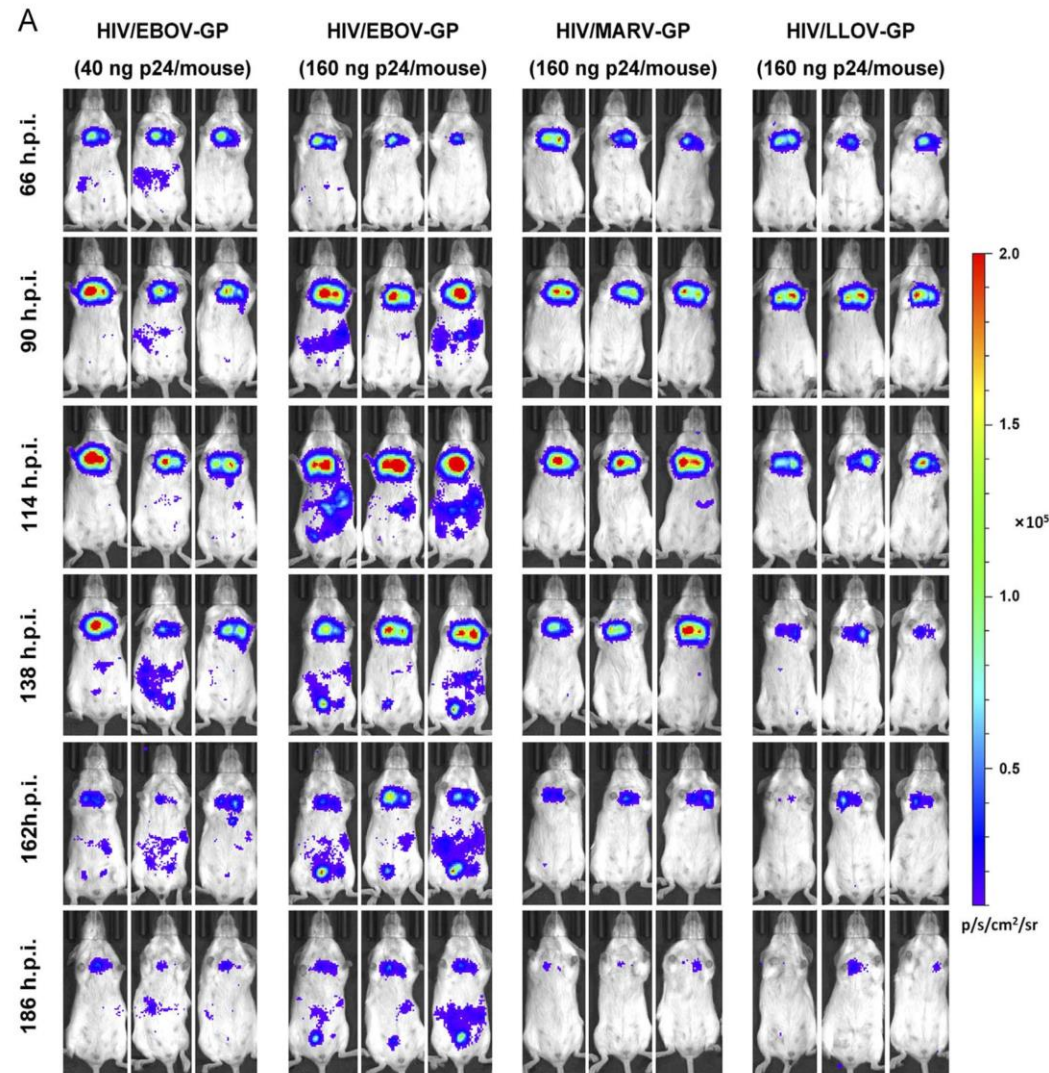
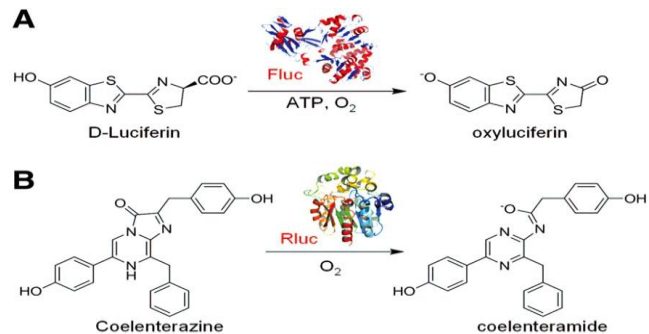
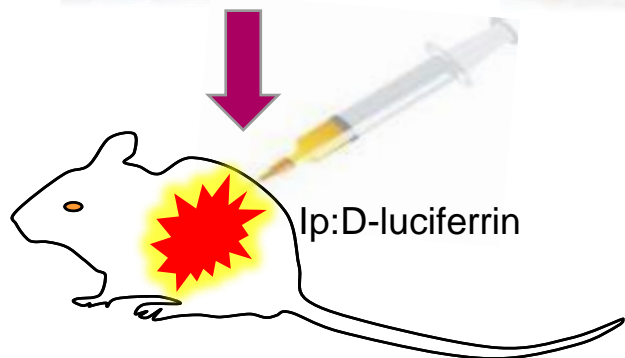
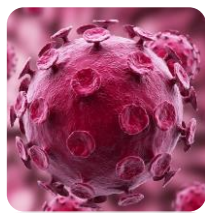
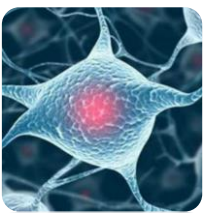
静脉注射



结肠炎模型



# 病毒感染的监测和治疗





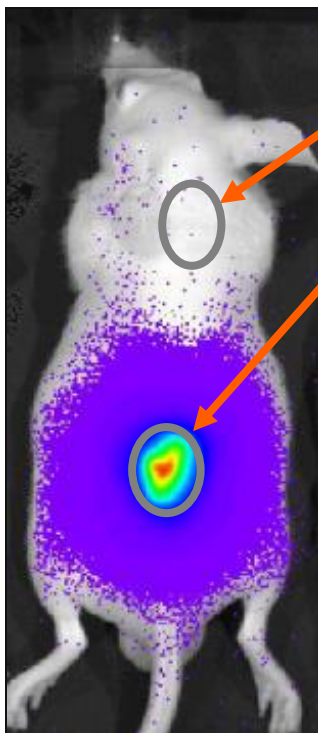
成像模式选择

荧光成像

生物发光成像

# 成像模式选择——生物发光 vs 荧光

生物发光成像

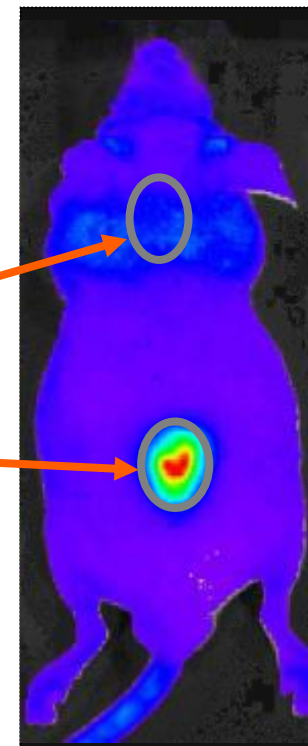


背景信号强度  $\sim 9.5 \times 10^3$  p/s  
光信号强度  $\sim 7.1 \times 10^7$  p/s  
信噪比  $\sim 7500$

背景信号强度  $\sim 1.0 \times 10^9$  p/s  
光信号强度  $\sim 7.8 \times 10^9$  p/s  
信噪比  $\sim 7.8$

$3 \times 10^6$  PC3M-luc/DsRed cells injected s.c.

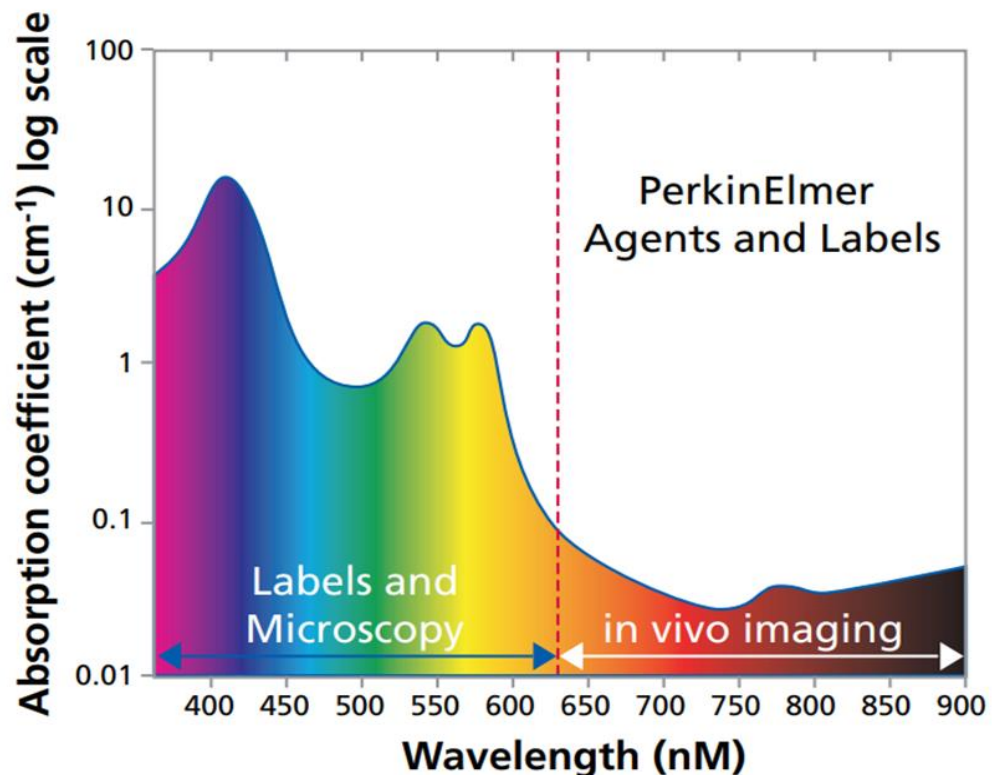
荧光成像



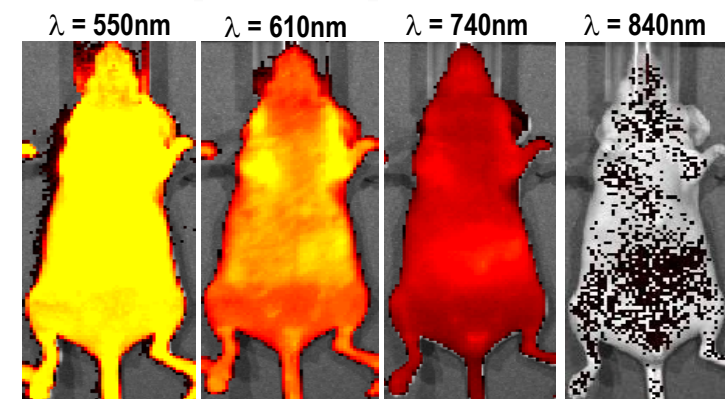
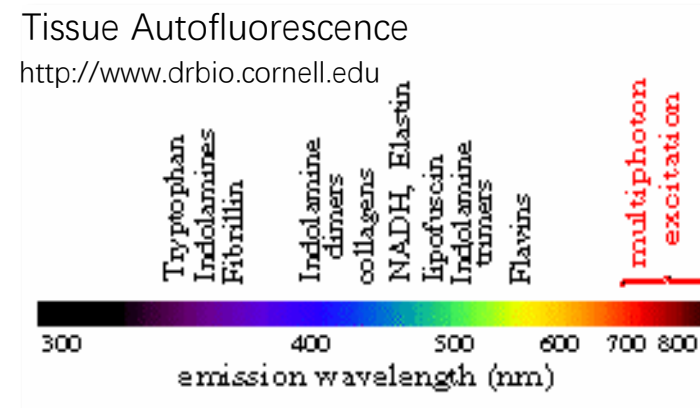
荧光成像灵敏度通常比生物发光成像低1000倍

# 荧光波长选择—透过率和背景噪音

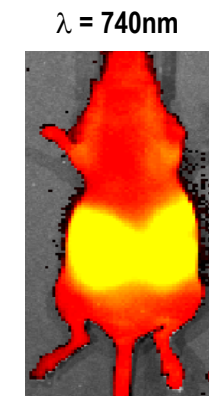
$$\text{SNR} = \text{Signal} / \text{Autofluorescence}$$



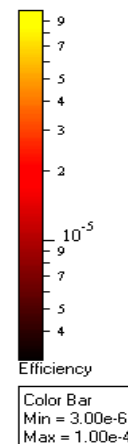
血红蛋白和其他机体组分强烈吸收可见光。  
在近红外区域，组织吸收显著降低。



Alfalfa Free Rodent Food

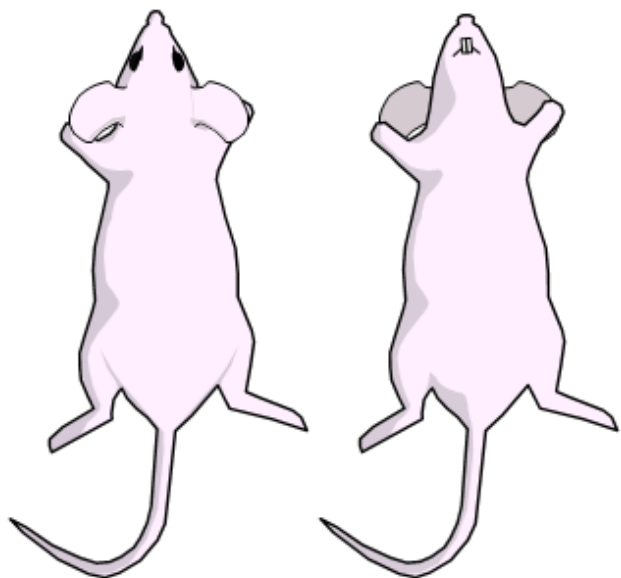


Regular Food



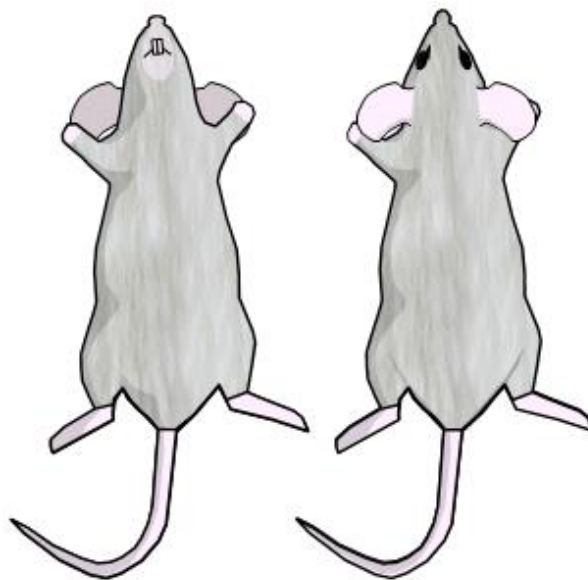


# 实验动物必须剃毛



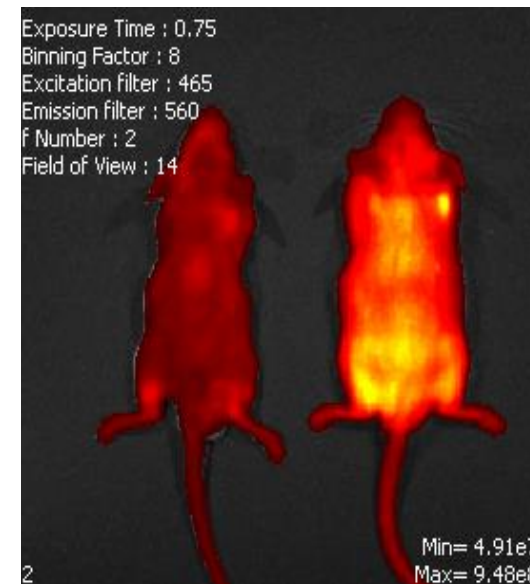
## Depilation not required

- Nude mice
- SKH1-E



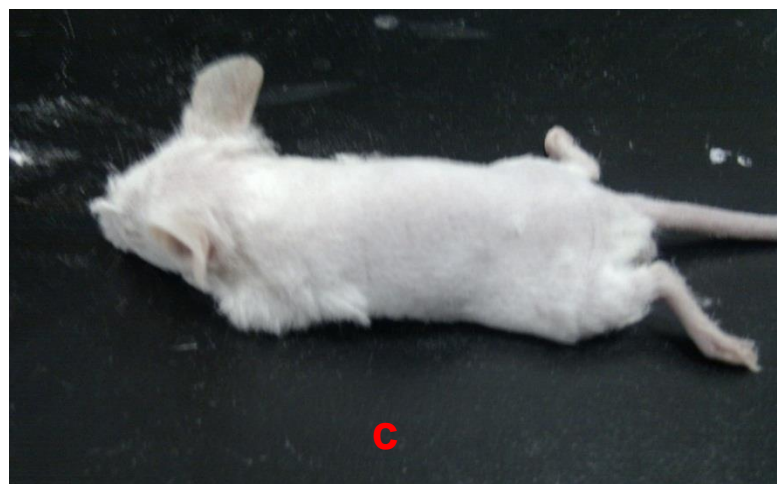
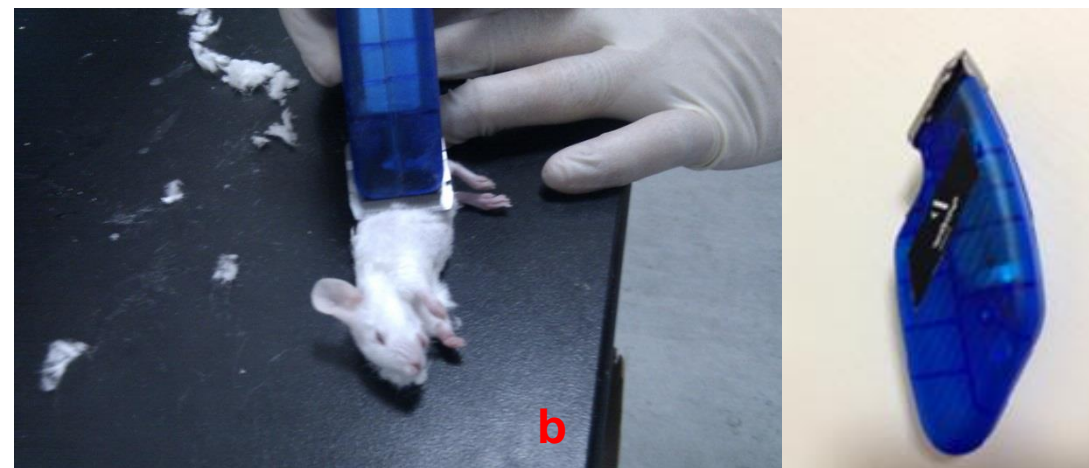
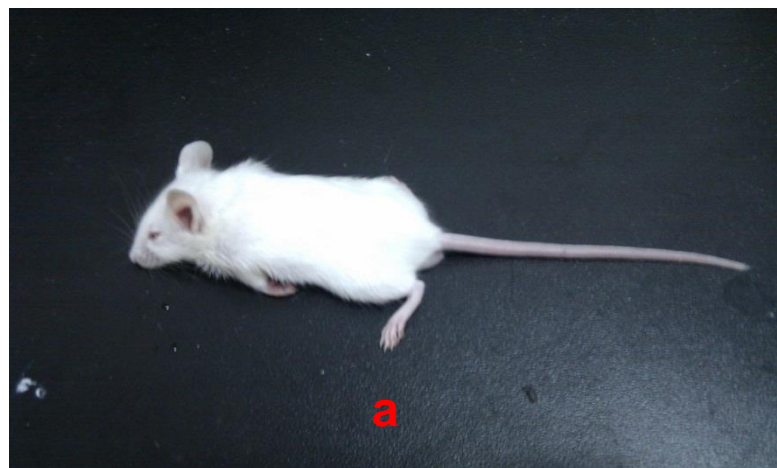
## Depilation required

Normal, haired mouse strains  
BALB/c, C57BL/6 etc . . .



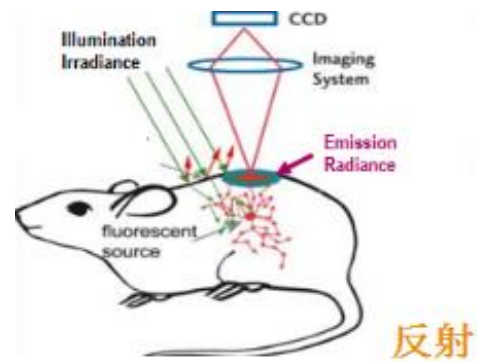
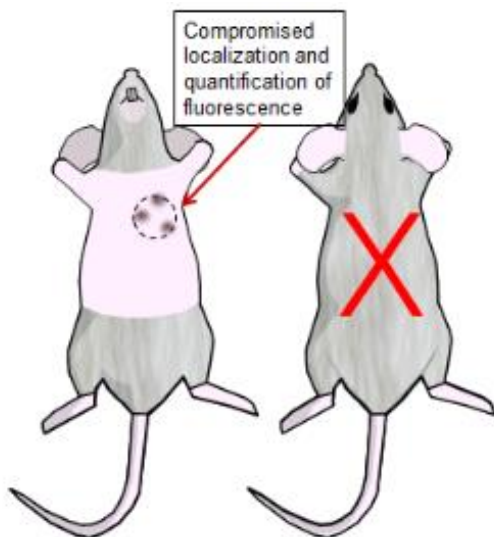
- 剃毛作用：降低毛发光吸收和光散射；去除来自毛发的背景荧光
- 实验前一天剃毛

# 剃（脱）毛方法

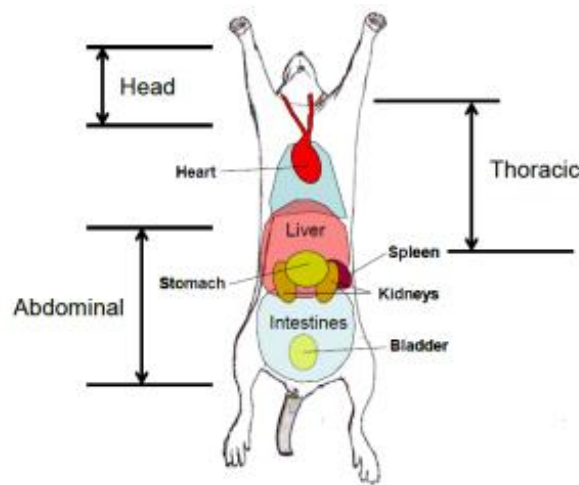
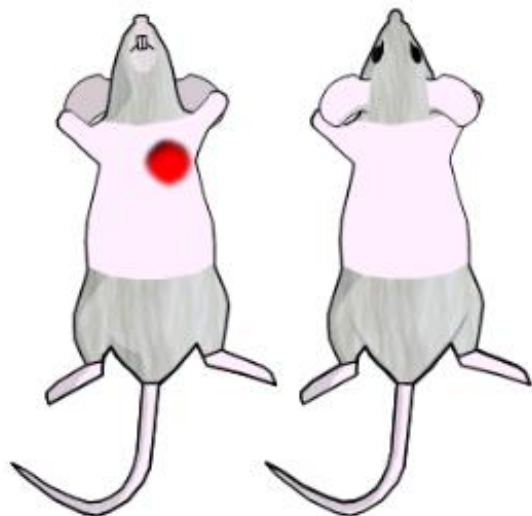


- a 剃毛前，体重16 g (约4 week) Balb/c 雄性小鼠；
- b 使用PerkinElmer剃毛器对小鼠进行剃毛；
- c 使用剃毛器后的效果，基于剃毛刀头的设计，有约1 mm的体表绒毛无法剃除；
- d 使用脱毛膏，可以将剩下的体表绒毛干净地去除，用完脱毛膏后用纱布蘸温水擦拭小鼠，避免脱毛膏的自发荧光干扰。

# 荧光成像小鼠剃毛——降低光吸收



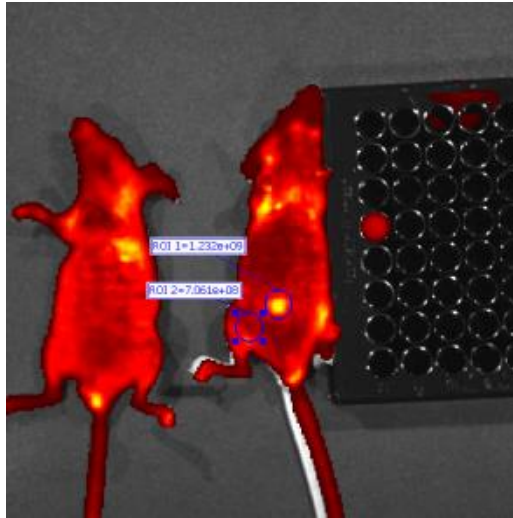
- 荧光反射成像时一般只需剔除成像面侧毛



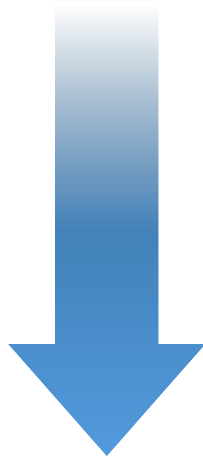
- 荧光透射成像时需剔除成像面两侧毛

# Spectrum成像模块——光谱拆分

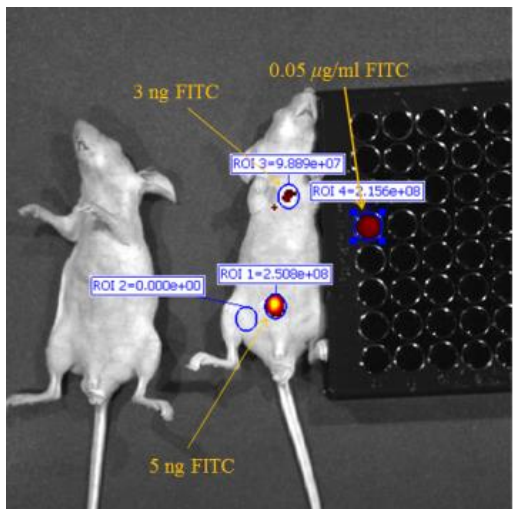
## 荧光成像的挑战——组织背景荧光



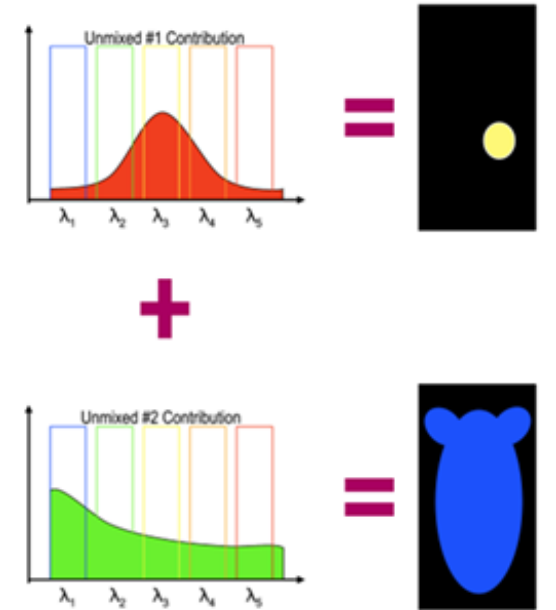
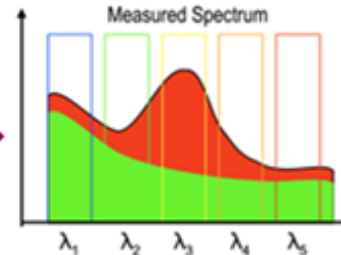
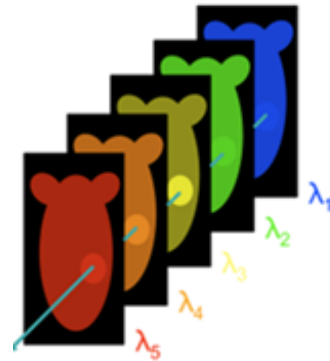
背景荧光



目标信号



## 光谱扫描硬件配置



(12) United States Patent  
Cronin et al.

(16) Patent No.: US 6,930,773 B2  
(45) Date of Patent: Aug. 16, 2005

(54) SPECTRAL IMAGING

(75) Inventors: Paul J. Cronin, Charlestown, MA (US); Peter J. Miller, Newburyport, MA (US)

(73) Assignee: Cambridge Research and Instrumentation, Inc., Woburn, MA (US)

(\*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 275 days.

(21) Appl. No.: 10226,692

(22) Filed: Aug. 23, 2002

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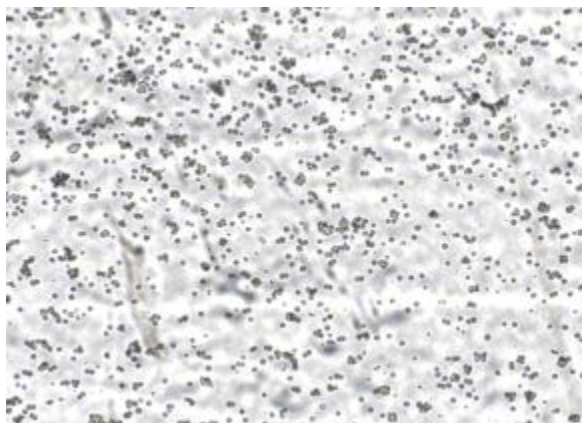
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IVIS系统具有**专利**的荧光光谱分离技术，使用该**技术**已发表超过**500篇文献**

# 细胞生物发光效率测试

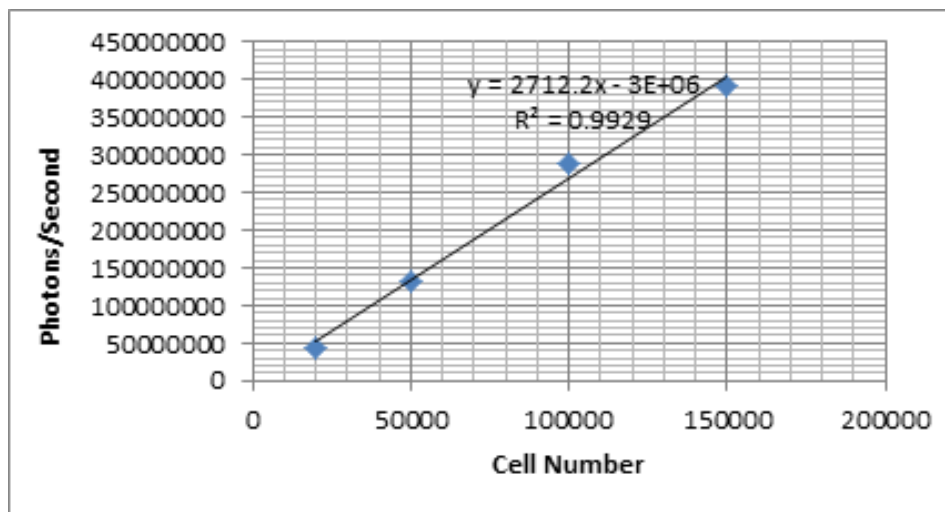
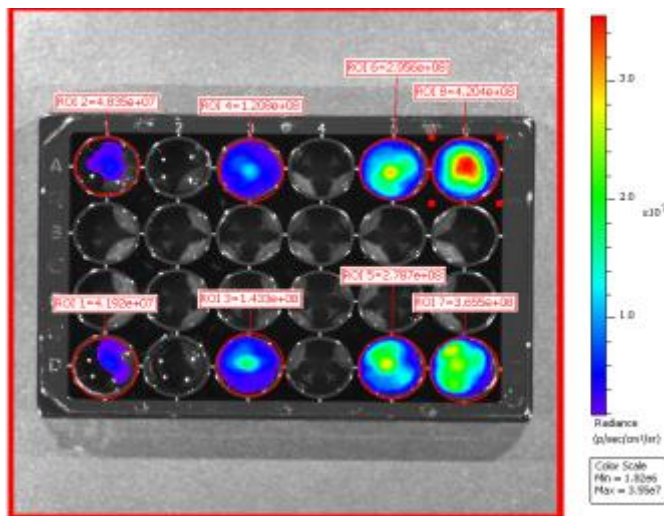
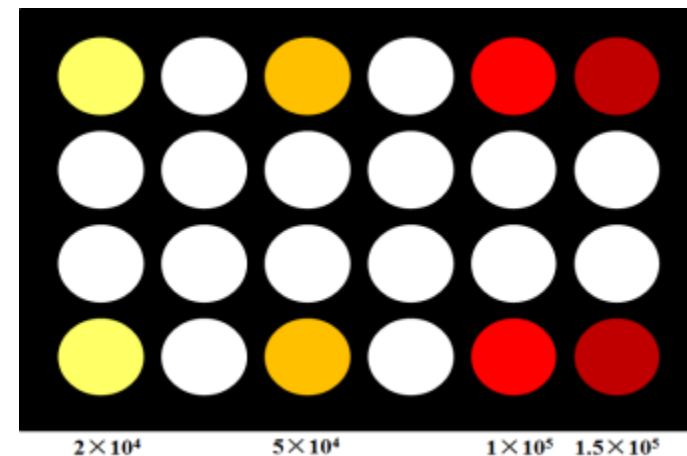
## 细胞计数



## PerkinElmer #1450-605



## 梯度细胞种板



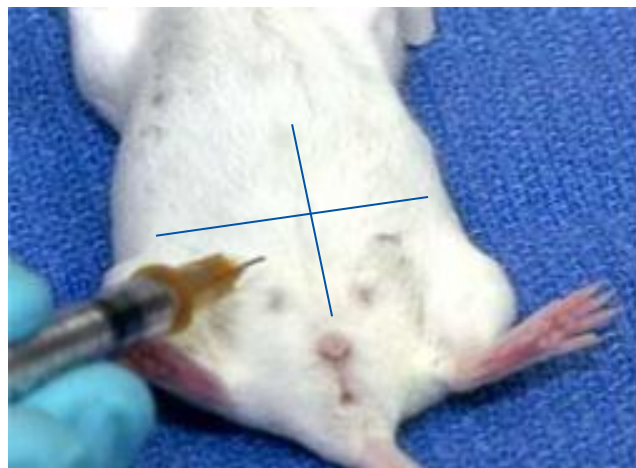
≥200

加入150  $\mu\text{g/ml}$  D-luciferin底物溶液  
3 min后使用IVIS系统成像

统计并计算Photons/sec/cell值

# 生物发光底物注射

## 腹腔注射



- 底物注射后 **10~20min** 进入成像平台期
- 2 h后信号消失

## 尾静脉注射

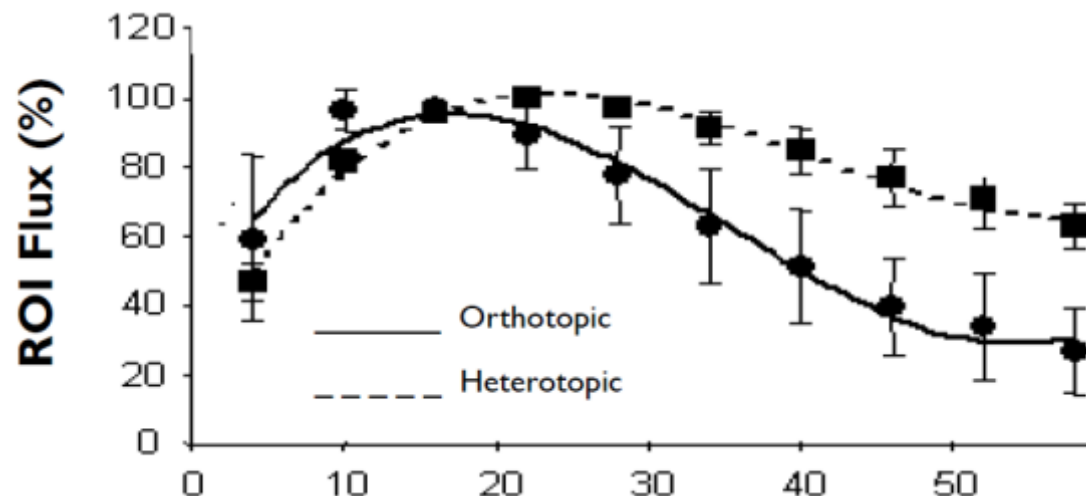


- 底物注射后约 **2min** 进入信号峰值
- 强度数倍于腹腔注射
- 约30min信号消失

底物剂量: **150 mg D-Luciferin/kg** 鼠体重

**!!!** 对每个生物发光实验动物模型, 首先需测一条**生物发光动力学曲线**, 得出最适成像时间点

1. 注射荧光素酶底物（建议在动物清醒时注射）；
2. 3 min后，麻醉动物（建议使用气体麻醉）；
3. 将麻醉后的动物放入成像仓中，在荧光素注射后约**5min**时刻拍摄第一张图片；
4. 之后**每2 min拍一张，持续拍摄60 min**，得到一条针对该实验模型的荧光素动力学曲线



原位和异位种植的Brain tumor  
的生物发光荧光素动力学曲线

- 依据上述曲线选取**最佳成像时间点**。
- 一般情况下，大多数实验模型的最佳成像时间点位于荧光素腹腔注射后**10-20分钟**。

1. 不要使用标记笔在小鼠身上做标记；建议剪脚趾或在小鼠尾巴尖端做标记，并用黑纸板盖住；
2. 尽量选择异氟烷气体麻醉的方式，避免使用液体注射麻醉；
3. 检测荧光弱信号，如果效果达不到预期，请尝试用黑纸板遮住其它有干扰的强信号后拍摄；
4. 如果看肿瘤转移等微弱的生物发光信号，需要把较强的原位肿瘤信号遮住；



## 运行环境

- 仪器工作时必须保证**室内温度 $\leq 26$ 度**（超过26度会加大像机的损耗）；
- 相对**湿度小于60%**；
- 室内**无明显扬尘**。

## 开关机注意事项

- 1) 短时间停机（停用间隔在数小时之内）：退出关闭Living image软件即可。
- 2) 下班停机：关闭软件，关闭电脑。
- 3) 假期停机：关闭软件，关闭电脑，关闭主机电源，关闭像机电源。

## 其他

- 文件路径全英文，勿出现中文路径
- 文件保存地址D盘，勿存C盘或桌面
- 为了防止电脑中病毒，不要在电脑上使用U盘。电脑上有刻录机，可以刻录光盘转移数据。
- 实验结束后，请将样本取出，并清理载物台，保持载物台干净。
- Spectrum型号每年至少检查一次仪器像机水冷散热系统是否缺液，如果需要及时补充冷却液。



*Thanks for your attention!*

Hanying.Du

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微信号：珀金埃尔默生命科学  
分享活体动物成像技术经验，介绍软件使用技巧，提供最新应用交流平台