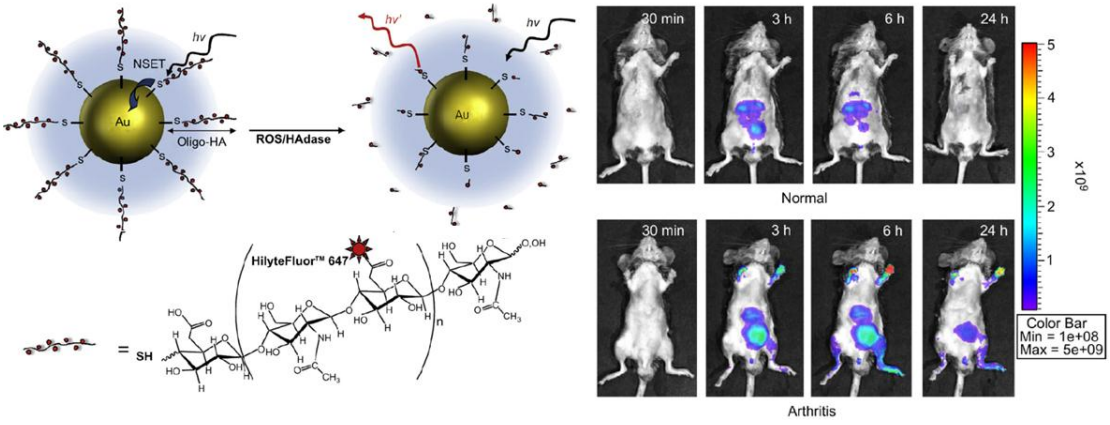
小动物活体光学成像技术在免疫学研究中的应用

Revvity小动物活体光学成像技术已在生命科学基础研究、临床前医学研究及药物研发等领域得到广泛应用。在众多应用领域中，免疫研究是活体光学成像技术的应用热点之一。在应用活体光学成像技术进行免疫学研究中，常用的标记方法及应用领域包括：1、利用功能性探针监测免疫疾病的发生发展及相关治疗；2、利用荧光素酶基因或荧光染料标记免疫细胞，监测免疫细胞的免疫应答作用；3、利用荧光素酶作为报告基因标记疾病相关基因构建转基因动物，进行免疫疾病机理研究。下面结合一些具体实例进行阐述：

一.监测免疫疾病的发生发展及治疗效果

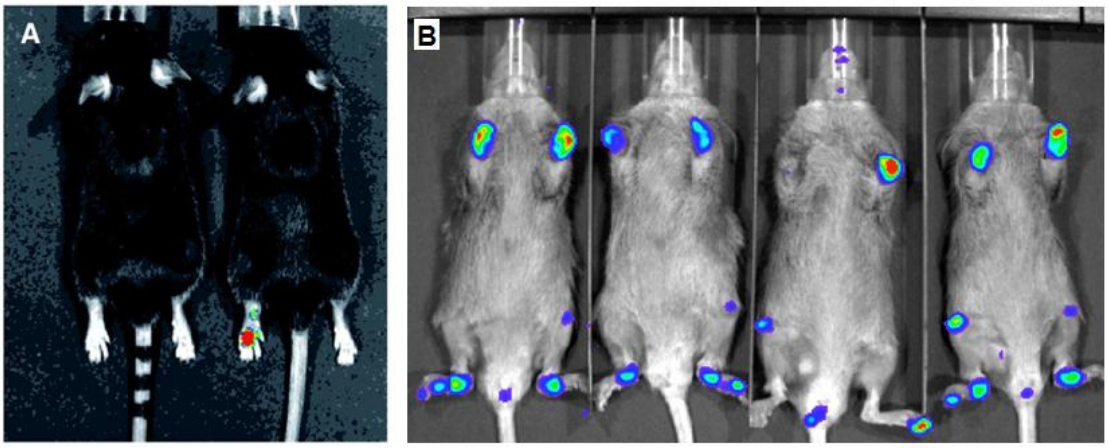
伴随免疫疾病研究的深入，目前已开发出一系列针对免疫疾病监测的功能性探针，这些探针的设计大多是基于在免疫疾病中表达的特征性分子或酶，通过对特征性分子或酶的监测而反映疾病的发生发展。利用这些探针并结合活体光学成像技术，研究者可以方便快捷地在活体动物水平监测免疫疾病的发生发展及治疗效果。

如在各种炎性疾病的发生发展中，通常会伴随大量活性氧自由基（ROS）及氧化胁迫的产生。研究者根据上述特征，设计出能够特异性探测 ROS 或氧化胁迫相关酶的功能性探针用于炎性疾病的检测。Lee 等应用其自行研发的表面结合透明质酸的金纳米颗粒（HA-AuNPs）成功检测了小鼠关节炎的发生。该纳米颗粒表面结合了荧光染料标记的透明质酸，当未被激活时，由于荧光染料及纳米颗粒本身的相互作用而处于荧光湮灭状态，而表面结合的透明质酸一旦被 ROS 或透明质酸酶剪切，便会被外界光源激发而发光。应用该探针并结合活体光学成像技术，便可在活体动物水平灵敏监测到炎性疾病的发生。

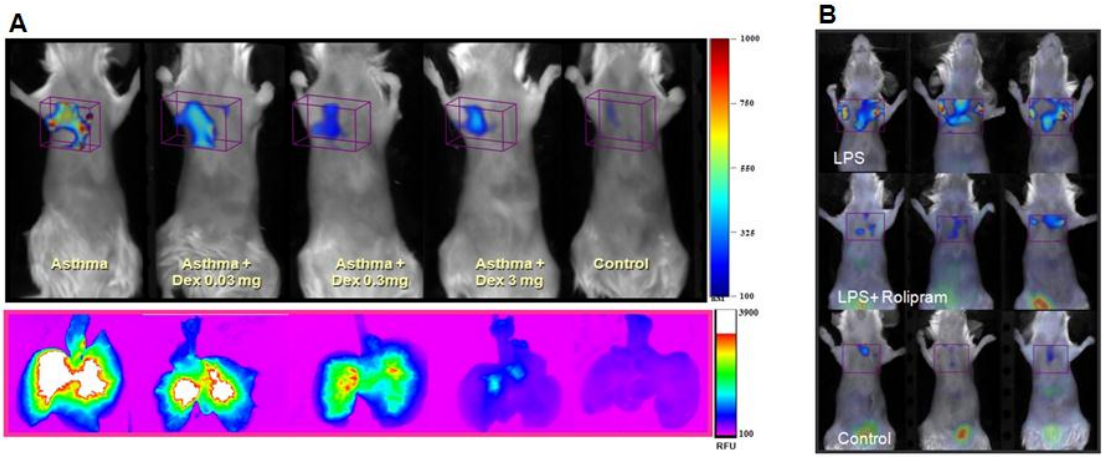


上图：应用 HA-AuNPs 及 IVIS 系统观测小鼠四肢关节炎的发生。尾静脉注射探针，不同时间点观测。

Revvity依靠强大的生物学研发团队，成功开发出多种应用于炎性疾病监测的功能性探针，如用于探测炎性细胞中髓过氧化物酶（MPO）的化学发光（Chemiluminescent）探针“XenoLight® RediJect™ Inflammation Probe”、用于探测环氧酶 2（COX-2）的近红外荧光探针“XenoLight® RediJect™ COX-2 Probe”、用于探测炎性细胞中组织蛋白酶（Cathepsin）的近红外探针“ProSense 680/750”、用于探测炎性细胞中基质金属蛋白酶（Matrix Metalloproteinase）的近红外探针“MMPSense 680/750”、用于探测嗜中性粒细胞胰肽酶（Neutrophil Elastase）的近红外探针“Neutrophil Elastase 680 FAST”。这些探针已被广泛应用于各种炎性疾病的研究中。



上图：应用 IVIS 成像系统及功能性探针监测小鼠关节炎的发生。A、利用 XenoLight® RediJect™ COX-2 探针观测关节炎发生过程中 COX-2 的表达；B、利用 XenoLight® RediJect™ Inflammation Probe 观测关节炎发生过程中 MPO 的表达。



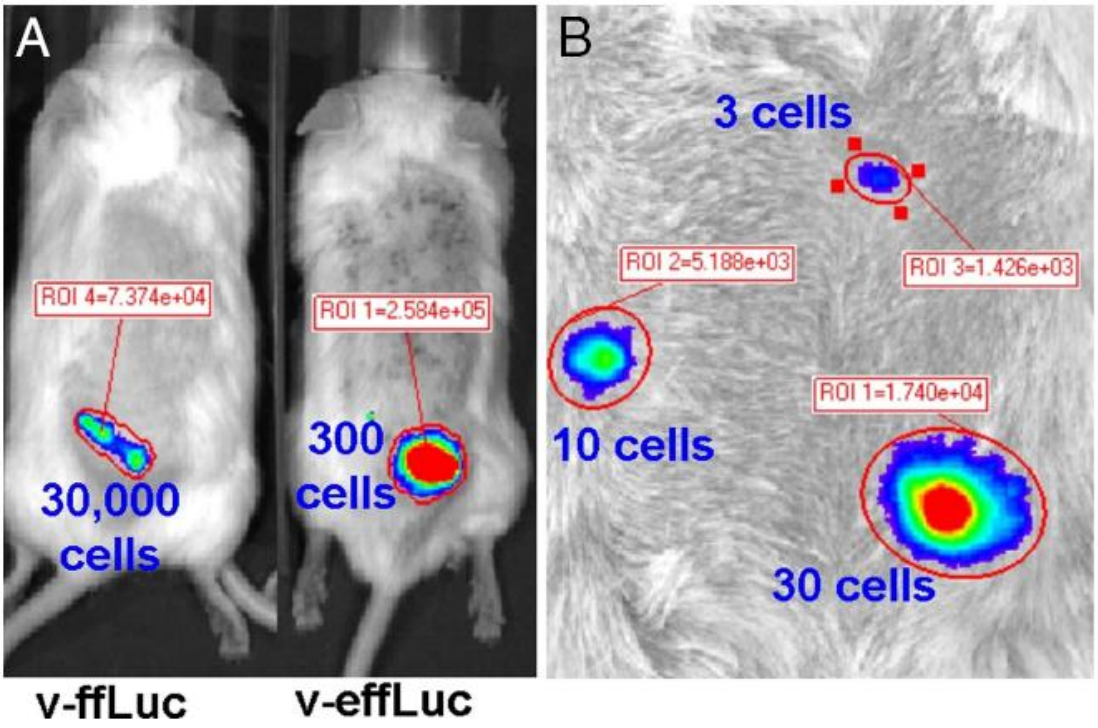
上图：应用 FMT 成像系统及功能性探针监测肺炎的发生及治疗效果。A、利用 ProSense 750 探针观测哮喘中组织蛋白酶的表达及地塞米松（dexamethasone）对哮喘的治疗效果；B、利用 Neutrophil Elastase 680 FAST 探针观测急性肺炎（COPD）的发生及 rolipram 的治疗效果。

二.监测免疫细胞的免疫应答

免疫应答是机体免疫系统对抗原刺激所产生的以排除抗原为目的的生理过程。这个过程是免疫系统各部分生理功能的综合体现，包括了抗原递呈、淋巴细胞活化、免疫分子形成及免疫效应发生等一系列的生理反应。通过有效的免疫应答，机体得以维护内环境的稳定。免疫细胞在机体的免疫应答中发挥着重要作用，了解免疫细胞的作用机理是免疫学研究的重要环节。活体光学成像技术已广泛应用于免疫细胞的相关研究，通过该技术可以在活体动物水平监测免疫细胞在相关疾病中的迁移、分布及功能。目前用于标记免疫细胞的主要方法包括：1、通过带有荧光素酶基因或荧光蛋白基因的病毒载体稳定转染人源或鼠源免疫细胞，使免疫细胞具有发光性质；2、直接从转基因发光小鼠中提取免疫细胞，所获得的免疫细胞即有发光性质；3、通过特定的荧光染料直接标记免疫细胞使其具有发光性质。研究者可根据具体研究，选择合适的标记方法对免疫细胞进行标记。

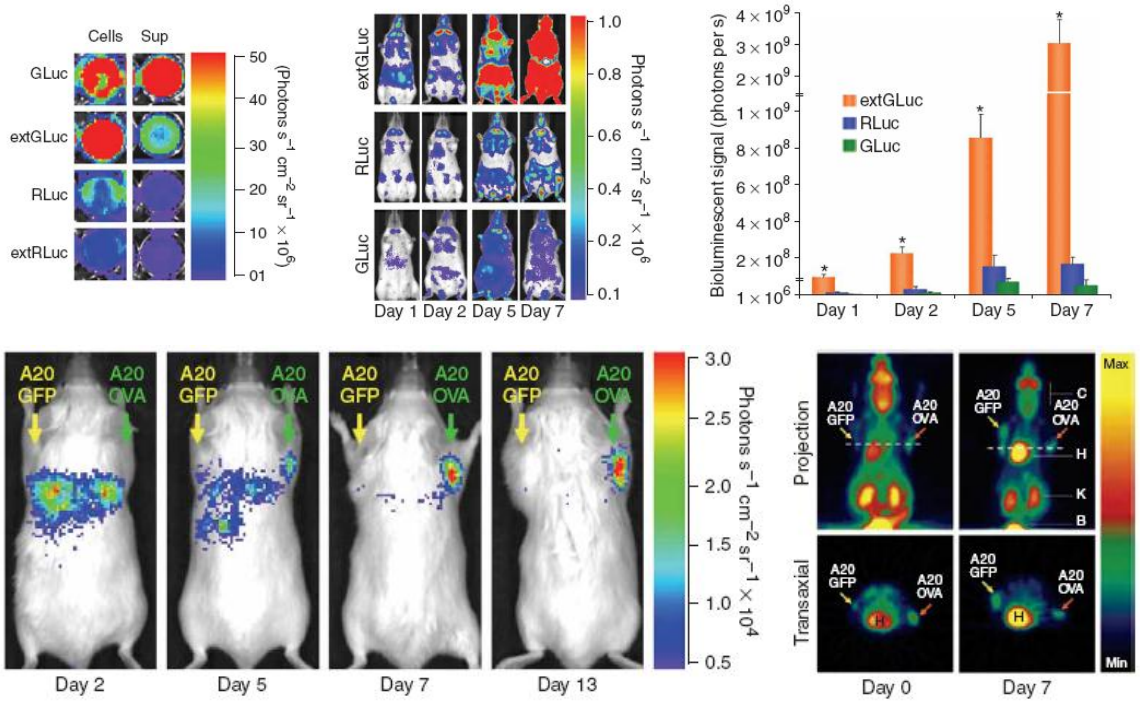
利用带有荧光素酶基因的病毒载体稳定转染免疫细胞的好处在于：1、生物发光灵敏度高，可以满足少量被标记免疫细胞的活体观测；2、由于是稳定转染，因此发光特性可以传代，不会因为细胞的分裂或分化而改变，可以进行数周或更长时间的观测。

如 Rabinovich 等人利用增强型萤火虫荧光素酶基因（effluc）标记 T 细胞，发现其发光强度比传统萤火虫荧光素酶（ffluc）高出至少 100 倍，将标记的 T 细胞皮下移植后，利用 IVIS 系统能够非常灵敏的观测到 3 个细胞发出的光信号。



上图：利用 IVIS 系统活体观测经荧光素酶基因标记的 T 细胞。A、分别利用 ffluc 或 effluc 标记 T 细胞的对比成像结果；B、利用 effluc 标记 T 细胞皮下移植成像结果。

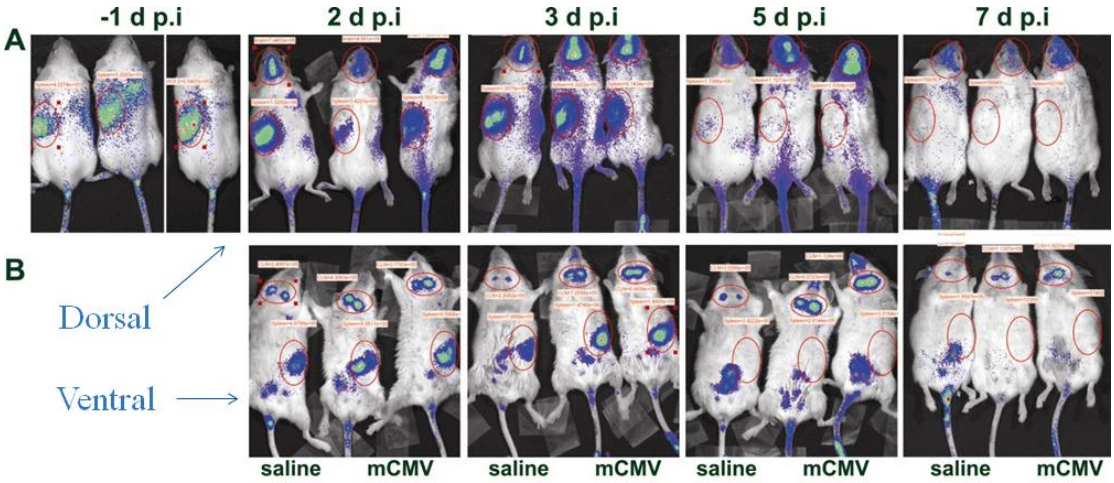
Santos 等利用桡足类动物膜结合型荧光素酶基因（ext GLuc）标记 T 细胞，发现其发光强度较萤火虫荧光素酶（ffluc）及海肾荧光素酶（Rluc）均有大幅提升。另外，研究者将 ext GLuc 标记的 T 细胞尾静脉注射入携带淋巴瘤 A20（OVA）的 SCID 小鼠体内，应用 IVIS 系统对 T 细胞在体内的分布进行长期观测，发现 T 细胞能够有效靶向肿瘤，并对肿瘤细胞具有杀伤作用。



上图：利用 IVIS 系统观测 ext GLuc 标记的 T 细胞。上行：体外及体内成像结果显示经 ext GLuc 标记的 T 细胞发光强度更高；下行：长期观测经 ext GLuc 标记的 T 细胞在体内的分布及对肿瘤的靶向治疗。

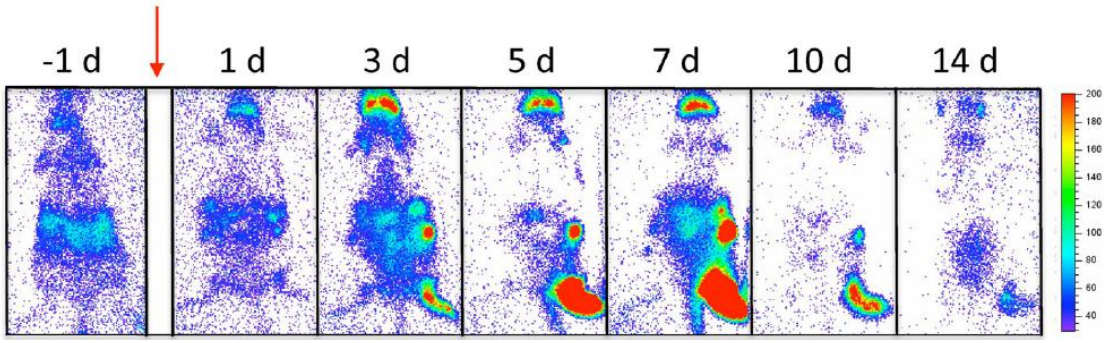
通过上述带有荧光素酶基因的病毒载体在体外转染免疫细胞，需要一定的实验基础及操作成熟度，并且与肿瘤细胞相比，免疫细胞的转染效率较低，没有成熟的实验操作经验较难获取荧光素酶高表达的转染细胞株。而通过从组成型（ubiquitous）表达或特异性（inducible）表达荧光素酶基因的转基因发光小鼠中提取免疫细胞的方法相对方便，无需自行进行标记，用提取的具有发光性质的免疫细胞即可开展实验。目前市场上已有多种商业化的发光转基因小鼠供研究者选择购买，如用组成型表达启动子控制荧光素酶基因表达而构建的转基因小鼠 Tg(β-actin-luc)和 Tg(CMV-luc)，其全身均有荧光素酶的表达，因此从脾、淋巴结、胸腺、骨髓等提取的免疫细胞即具备发光性质。

Cheeran 等利用从转基因小鼠 Tg(β-actin-luc)中提取的脾细胞及淋巴结细胞，研究了免疫细胞对病毒感染的响应。研究者将提取的发光脾细胞及淋巴结细胞通过尾静脉注入脑室内感染巨细胞病毒的小鼠，利用 IVIS 系统观测了上述免疫细胞在活体动物体内对感染病灶点的浸润。结果显示，在未经病毒感染的正常小鼠体内，移植的淋巴细胞主要聚集于脾内（如下图-1dpi 所示），而当小鼠脑部感染病毒后，这些淋巴细胞会迁移至感染区域而发挥免疫作用。



上图：利用 IVIS 系统观测免疫细胞对病毒感染的免疫应答。A、小鼠背部朝上拍摄；B、小鼠腹部朝上拍摄。-1dpi 为病毒感染前 24h 尾静脉注射淋巴细胞成像结果，每张图中从左至右第一只小鼠为注射生理盐水的对照小鼠，第二、三只小鼠为脑室内感染巨细胞病毒的疾病小鼠。

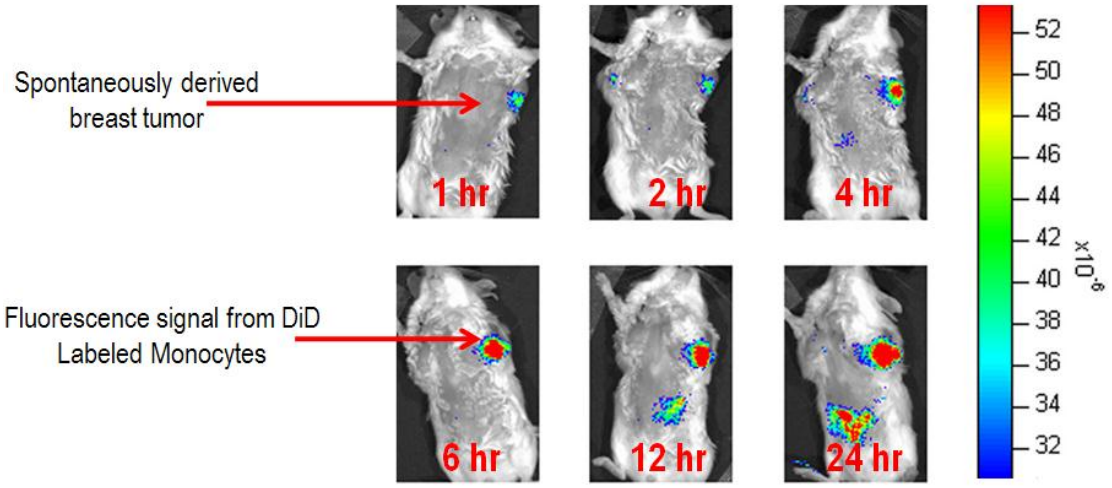
Chewning 等构建了利用 T 细胞特异性表达的人 CD2 基因启动子控制荧光素酶基因表达的表达载体，并应用该载体构建出转基因小鼠，从该转基因小鼠中提取的发光细胞即为 T 细胞。研究者将提取的发光 T 细胞经尾静脉注入由 OVA 诱导而引发炎症的小鼠体内，应用 IVIS 系统观测 T 细胞的免疫应答。



上图：利用 IVIS 系统观测 T 细胞对抗原的免疫应答。

除了上述两种通过荧光素酶基因对免疫细胞进行标记的方法之外，通过膜嵌合型荧光染料标记免疫细胞的方法则更为简便，研究者只需依据染料标记的实验流程，即可自行完成对免疫细胞的标记。目前市场上主要的膜嵌合型荧光染料包括 DiD、DiL、DiR 等，它们均是通过自身亲脂性的长碳链插入目标细胞的细胞膜中而进行标记。使用此种方式进行标记的好处在于操作相对简单，但由于是膜嵌合性标记，因此被标记细胞的发光性质不会传代，只能用于观测免疫细胞在体内的短时间动态变化。

之前的研究显示，乳腺癌的发生发展通常伴随有肿瘤周边的炎症发生，而炎性细胞在肿瘤周边炎症区域的聚集是炎症发生的必要条件。Sista 等利用 DiD 染料体外标记了单核细胞，并利用 IVIS 系统观测了尾静脉注射的单核细胞对原发性乳腺癌的靶向聚集情况。



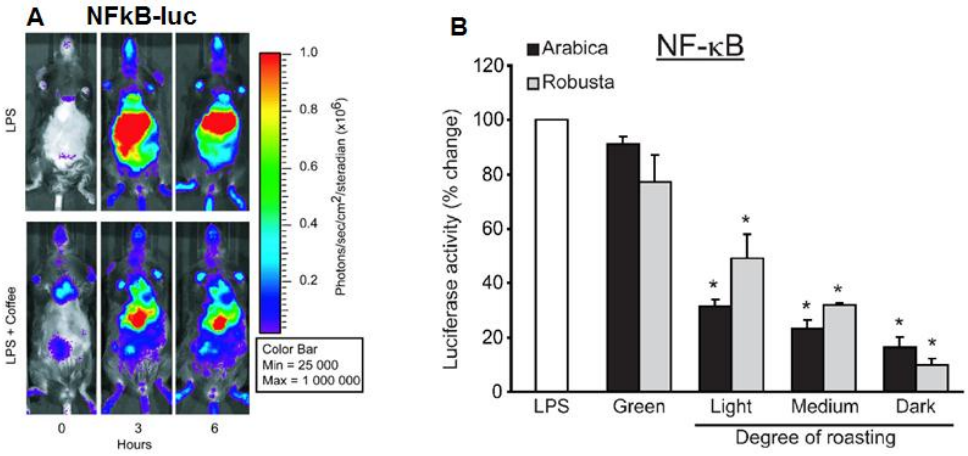
上图：利用 IVIS 系统观测经 DiD 染料标记的单核细胞对肿瘤的靶向聚集。单核细胞经尾静脉注射后不同时间点的成像结果，所用小鼠为乳腺癌原发性模型小鼠 MMTV-PymT。

三.免疫疾病机理研究

对于炎性相关基因的研究，可以揭示免疫疾病的分子机理，更好的了解免疫疾病的发生发展及相关治疗。小动物活体光学成像技术已越来越多的应用于此类研究。研究者通过构建各种生物发光转基因动物，结合活体光学成像技术，在活体动物水平观测免疫疾病发展过程中相关基因的表达。目前市场上已有不少成熟的可用于炎症研究的生物发光转基因小鼠，这些小鼠的构建通常是用炎性相关基因的启动子特异性控制荧光素酶基因的表达，从而通过发光情况反映炎症的发生及治疗效果。下图列举了几种可应用于相关免疫疾病研究的生物发光转基因小鼠模型，研究者可以从美国 Taconic 公司购买。

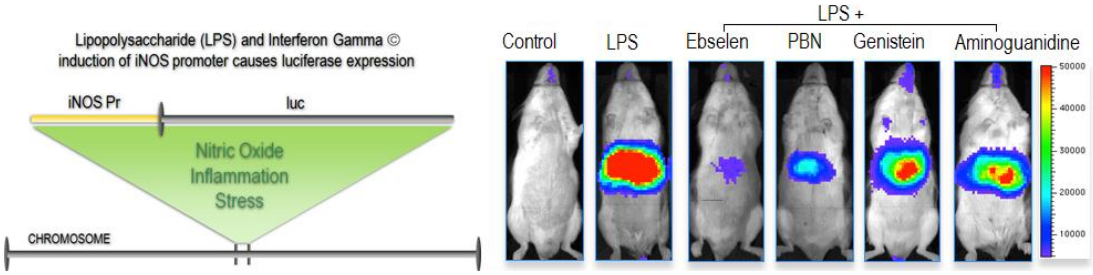
Disease	LPTA
❑ Rheumatoid Arthritis	NFkB RE, iNOS, SAA-1
❑ Neuro-inflammatory Disease	GFAP, SAA-1, COX-2
❑ Sepsis	NFkB-RE, IkB, SAA-1
❑ IBD	pS2/TFF-1, TNF-alpha
❑ Diabetes mellitus	RIP, Ins-2
❑ Pain	GFAP, Cox-2
❑ Asthma	Epx, NFkB RE

NFκB 是一类重要的转录因子，参与免疫反应的早期和炎症反应各阶段的许多分子都受 NF-κB 的调控，因此，对于 NFκB 信号通路的研究是炎症疾病研究的一个热点。Paur 等利用 Tg(NFκB-RE-luc)转基因小鼠，观测了咖啡对 LPS 诱导的炎症的抑制作用。结果显示，咖啡能够抑制 NFκB 的表达，并激活机体的抗氧化防御，而且随着咖啡烘焙程度的提高，这种抑制效应越明显。因此，利用 Tg(NFκB-RE-luc)转基因小鼠可以观测炎症的发生发展及相关药物的治疗效果。



上图：利用 IVIS 系统观测咖啡对 NFκB 表达的抑制。A、活体成像结果：上行为未给咖啡的对照组，下行为给咖啡组；B、不同烘焙程度咖啡对 NFκB 表达抑制的定量结果。

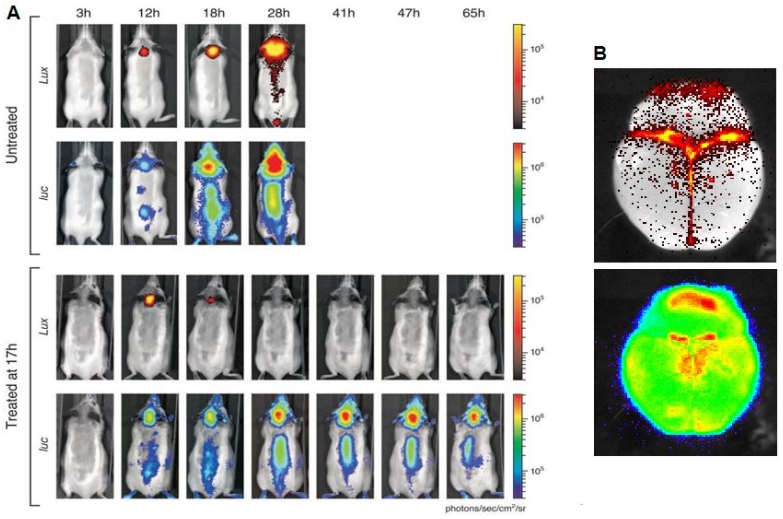
一氧化氮（NO）是细胞内的一种具有生物活性的重要调节分子，它既是是组织损伤的诱发因子和各种病变的增强因子，也是免疫应答的调节性分子。在炎性条件下，体内 NO 主要由炎性细胞中的诱导性一氧化氮合成酶（iNOS）产生，在败血症、关节炎、心血管疾病等多种炎性疾病中起调节作用。LPS 及多种细胞因子如干扰素 IFN-γ 等可激活 iNOS 的表达。下图所示为应用 Tg(iNos-luc)转基因小鼠观测不同抗炎药物对 iNOS 表达的抑制效应。研究者利用 LPS 诱导炎症的发生，对 iNOS 特异性表达的转基因小鼠施加不同抗炎药物，应用 IVIS 系统观测了不同抗炎药物对 iNOS 表达的抑制效果。



上图：利用 IVIS 系统及 Tg(iNos-luc)转基因小鼠观测不同抗炎药物对 iNOS 表达的抑制效果。

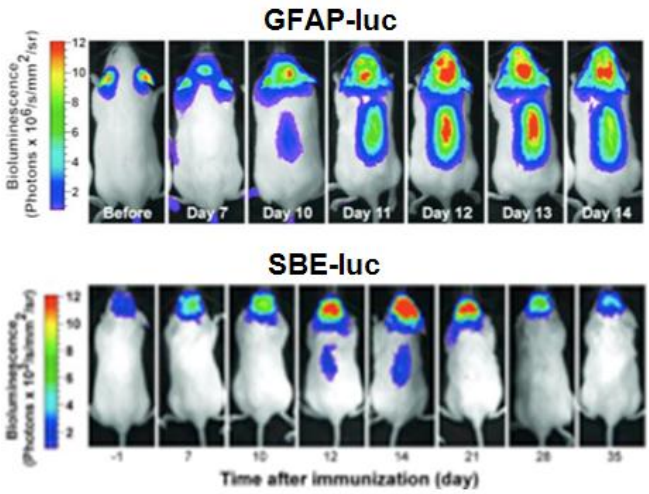
Cordeau 等利用 Tg(GFAP-luc)转基因小鼠，监测 GFAP 在肺炎链球菌感染而引发的脑膜炎中的表达。研究者用细菌荧光素酶（bacterial luciferase）基因标记肺炎链球菌，以监测细菌在活体动物体内的感染情况，同时以萤火虫荧光素酶基因标记的 Tg(GFAP-luc)转基因小鼠为实验动物，观测 GFAP 在肺炎链球菌感染而引发的脑膜炎中的表达，以及经抗生素治疗后细菌的感染情况和 GFAP 的表达情况。结果显示，随着细

菌感染程度及范围的升高和扩大，GFAP 的表达量也相应升高；而经抗生素治疗后，细菌的感染情况明显被抑制，GFAP 的表达量也随之降低。对小鼠感染脑部的体外成像结果显示，细菌对于脑部局部区域的感染，能引发整个脑部 GFAP 的大量表达。



上图：应用 IVIS 系统观测肺炎链球菌在 Tg(GFAP-luc)转基因小鼠中的感染情况及 GFAP 的表达情况。A. 活体成像结果，untreated：未经抗生素治疗，treated：经抗生素治疗；B.脑部体外成像结果。

Luo 等利用 Tg(GFAP-luc)及 Tg(SBE-luc) 转基因小鼠，结合活体光学成像技术，观测了在自身免疫性脑脊髓炎（EAE）中与 GFAP 表达相关的星形胶质细胞的聚集及与 TGF-β 信号通路相关的炎症的发生。研究者用髓磷脂少突细胞糖蛋白（MOG）免疫小鼠，引发实验性自身免疫性脑脊髓炎，随后，利用 IVIS 系统观测患病 Tg(GFAP-luc)及 Tg(SBE-luc)转基因小鼠中 GFAP 及 TGF-β 的表达情况。结果显示，小鼠免疫后的第 7 天，即可观测到 GFAP 及 TGF-β 表达量显著升高，说明在脑脊髓炎的发病初期，即伴随有星形胶质细胞的聚集以及炎症的发生。值得注意的是，脑脊髓炎的明显临床症状出现于免疫后 11 天，因此，与观察临床症状而诊断疾病发生的方法相比，通过应用活体光学成像技术观测疾病相关基因的表达，能够更早的观测到疾病的发生。



上图：应用 IVIS 系统观测自身免疫性脑脊髓炎小鼠中 GFAP 及 TGF-β 的表达情况。（上）应用 Tg(GFAP-luc)转基因小鼠观测 GFAP 的表达；（下）应用 Tg(SBE-luc)转基因小鼠观测 TGF-β 的表达。

IVIS APPLICATIONS IN IMMUNOLOGY RESEARCH

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