Literature Report 3

Fang Xiangning

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A Dual Fluorescence-Spin Label Probe for Visualization and Quantification of Target Molecules in Tissue by Multiplexed FLIM – EPR Spectroscopy

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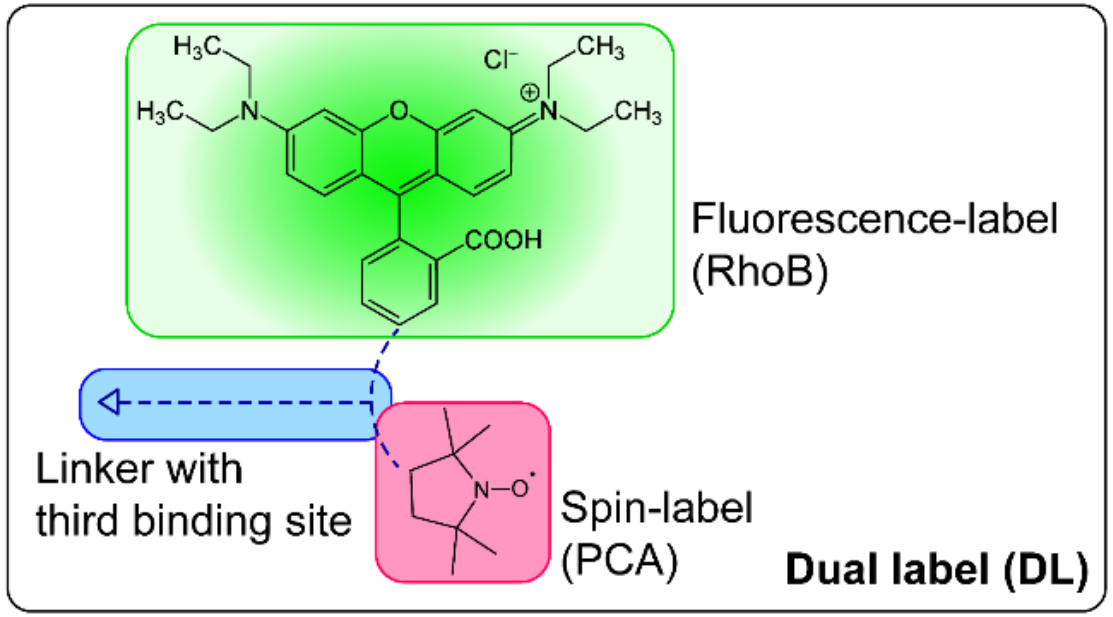

Multiparameter Read-Out

目标的可视化、量化

- Intensity
- Fluorescence Lifetime (concentration dependent)

荧光寿命成像显微镜（FLIM）

- Spin number
- Environment

电子顺磁共振光谱（EPR）

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Freie Universität Berlin 德国柏林自由大学

Martina Meinke

1. 血液和皮肤的光谱学
2. 皮肤中的电子顺磁共振（EPR）测量

Marcelo Calderón

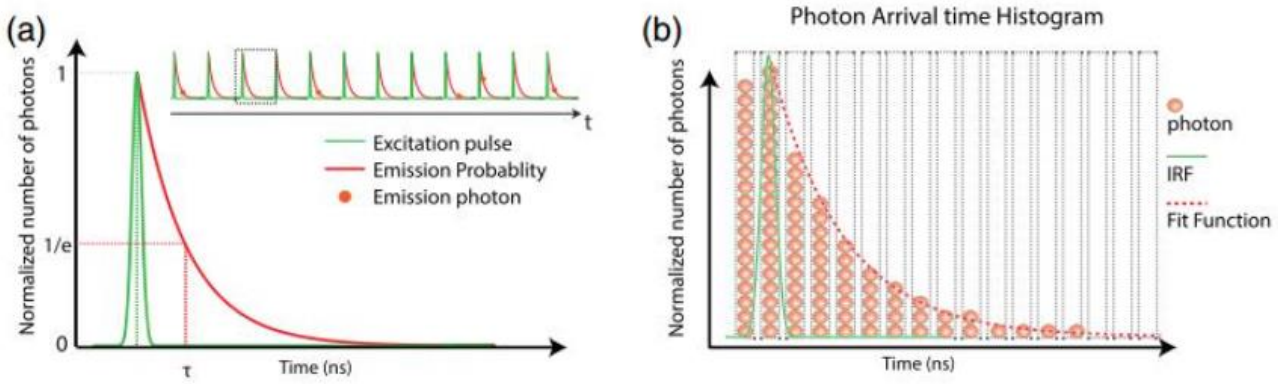
自组装聚合物、有机化学

1. 多功能聚合物-药物偶联物
2. 用于递送生物活性物质的两亲自组装分子
3. 作为诊断方法的新诊断技术

Background

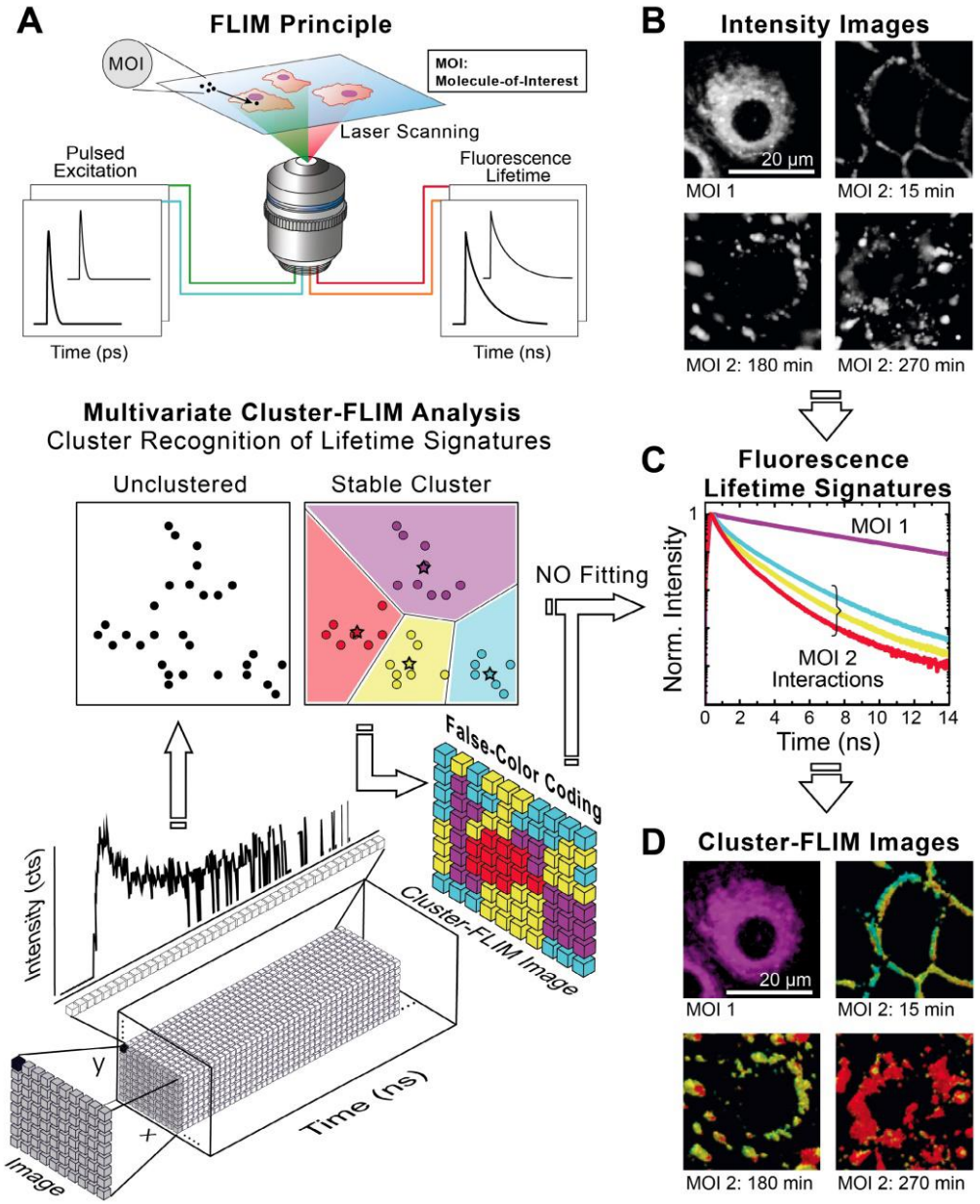
荧光寿命成像显微镜（FLIM）

- 时间相关单光子计数（TCSPC）
- Cluster信号提取



TCSPC：时域测量方法，测量荧光的发射几率随时间的变化。

J Biomed Opt., 2020, 7, 1
Theranostics, 2020, 6322

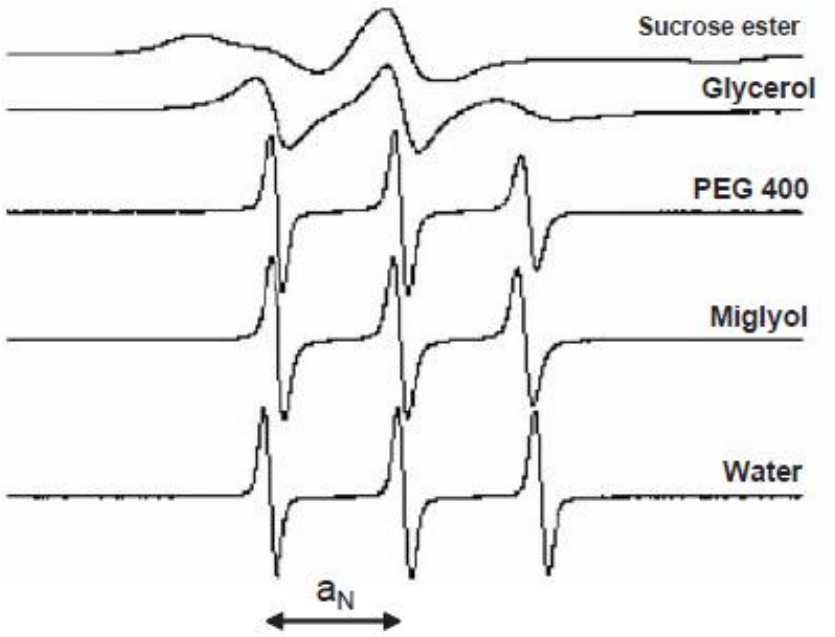


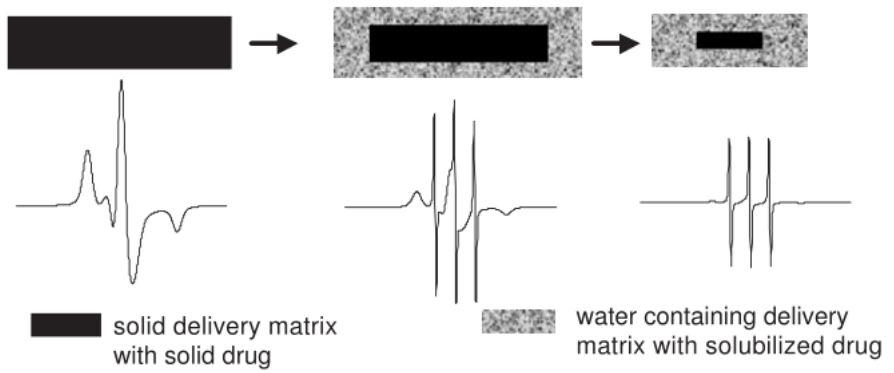
Background

电子顺磁光谱（EPR）

顺磁性：分子的外层轨道上含有未配对电子
顺磁性物质：自由基、过渡金属

- 定量分析组织中自旋标记分子的绝对数量，无需破坏性的提取程序
- 根据EPR信号的光谱形状、超精细耦合常数和旋转相关时间等参数可确定自旋标记分子周围微环境的信息，如极性、pH、粘度等

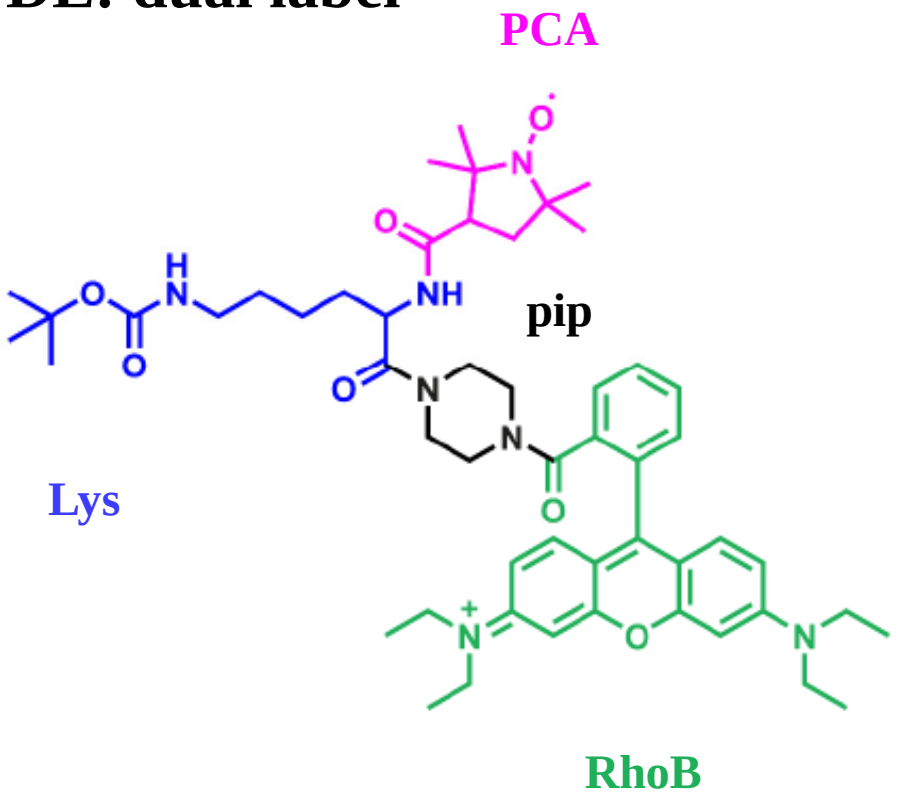




Advanced Drug Delivery Reviews, 2005, 1171

Probe

DL: dual label



RohB：荧光寿命和强度成像

PCA：商业化的自旋标记物

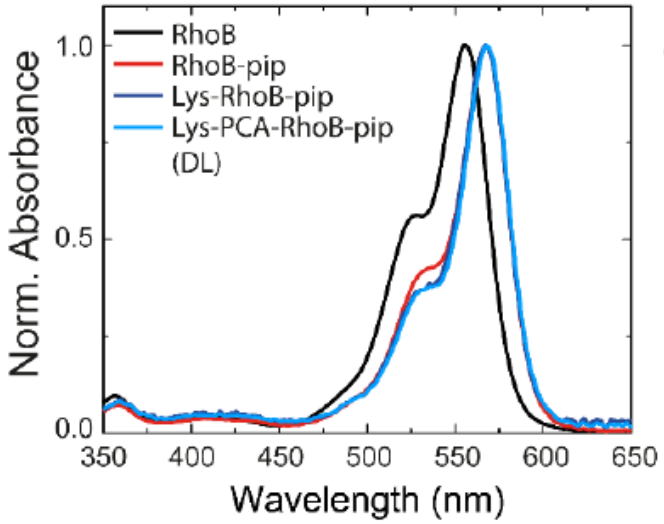
Lys：脱保护后提供与生物分子标记的位点

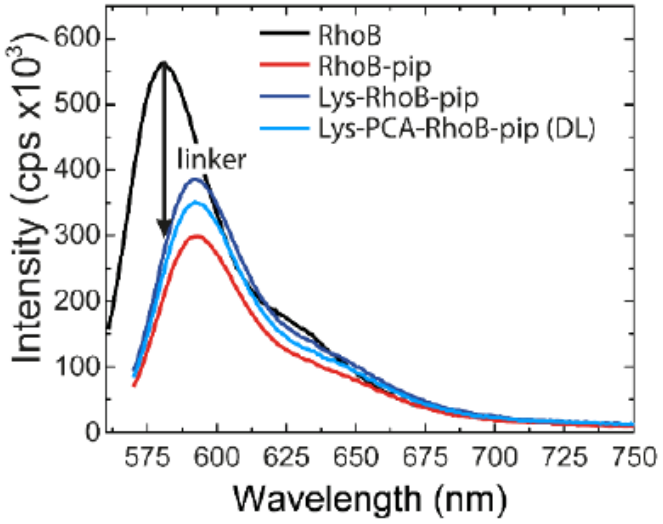
用途

- 由EPR和FLIM进行生物分子的组织分布可视化、浓度量化和微环境判断
- 皮肤组织中的药物渗透原理证明实验，对皮肤肿瘤抗癌药物的开发进行高空间分辨率和对浓度、环境变化的光谱分析

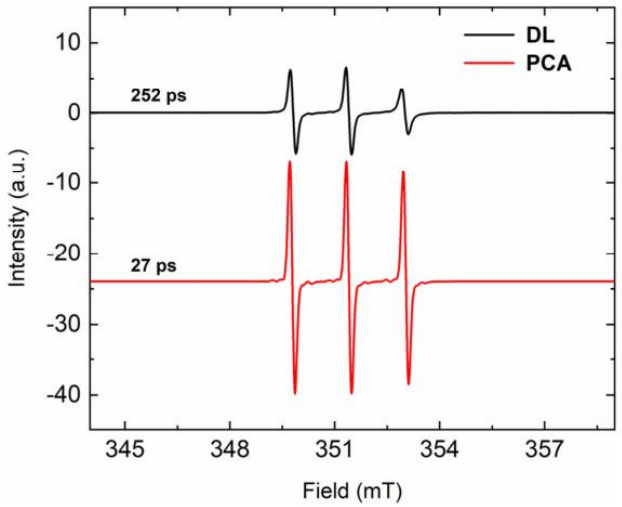
Probe

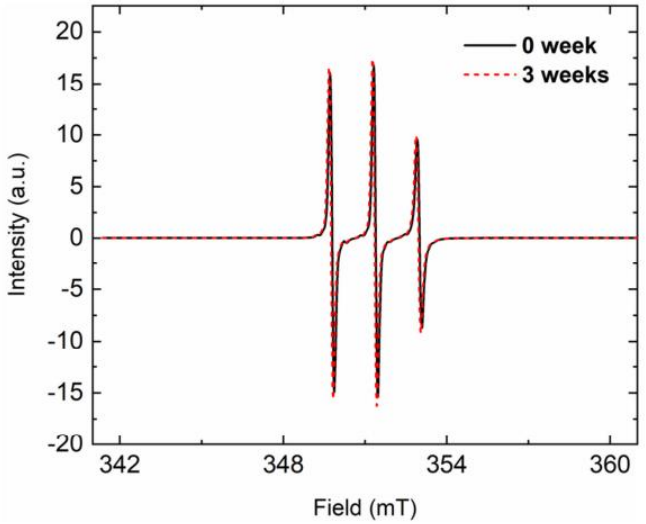
pip的取代对探针的光谱产生了较大的影响





Sample	$\bar{\tau}_{pop}$(ns)
RhoB	1.700±0.004
RhoB	1.69±0.01
RhoB-pip (**5**)	1.488±0.001
Lys-RhoB-pip	1.470±0.005
Lys-PCA-RhoB-pip (**6**)	1.412±0.002

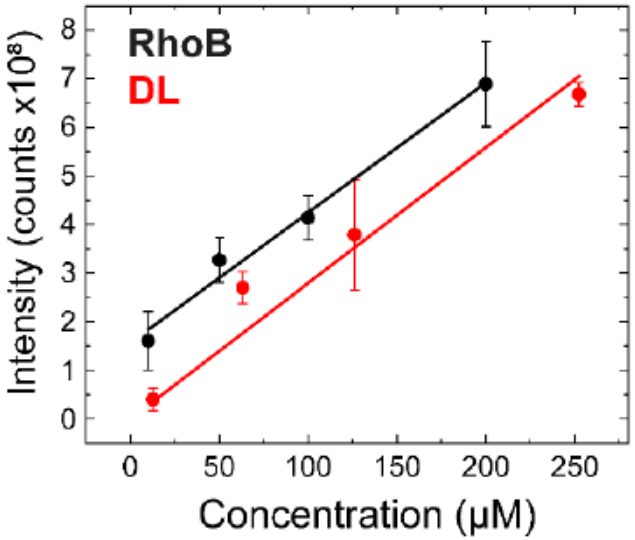


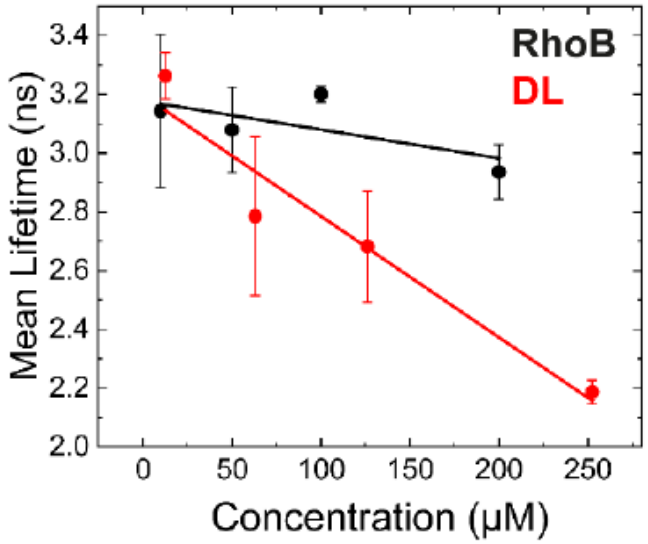


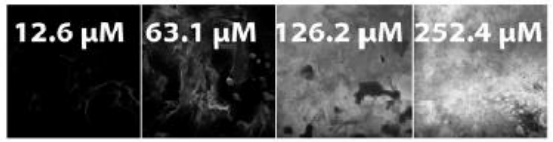
60%的DL探针具顺磁性

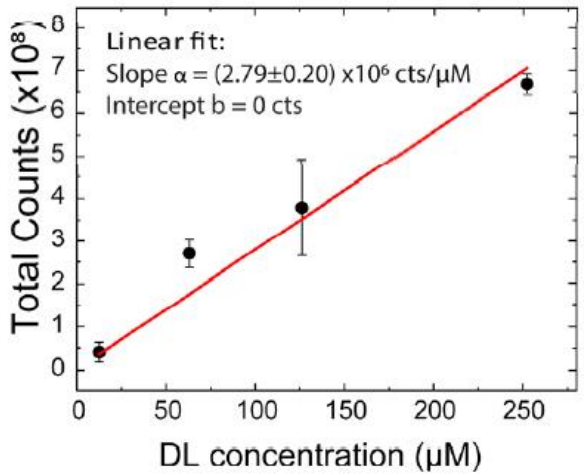
DL探针具有很高的稳定性

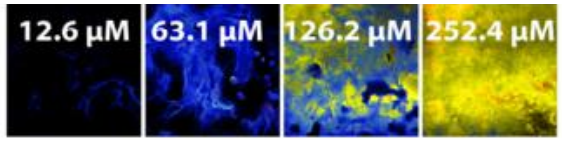
Experiments in tissue mimetics

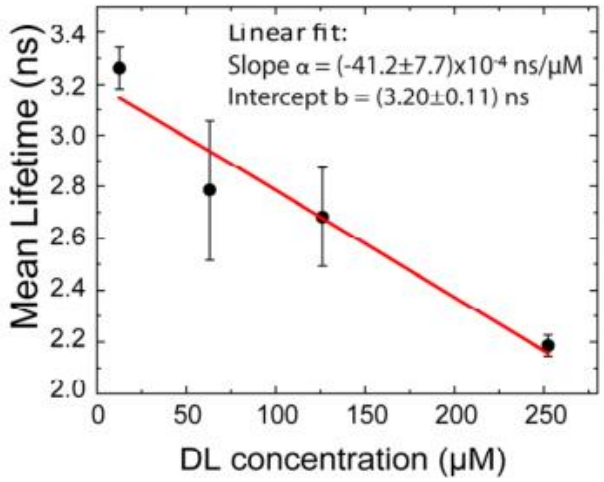










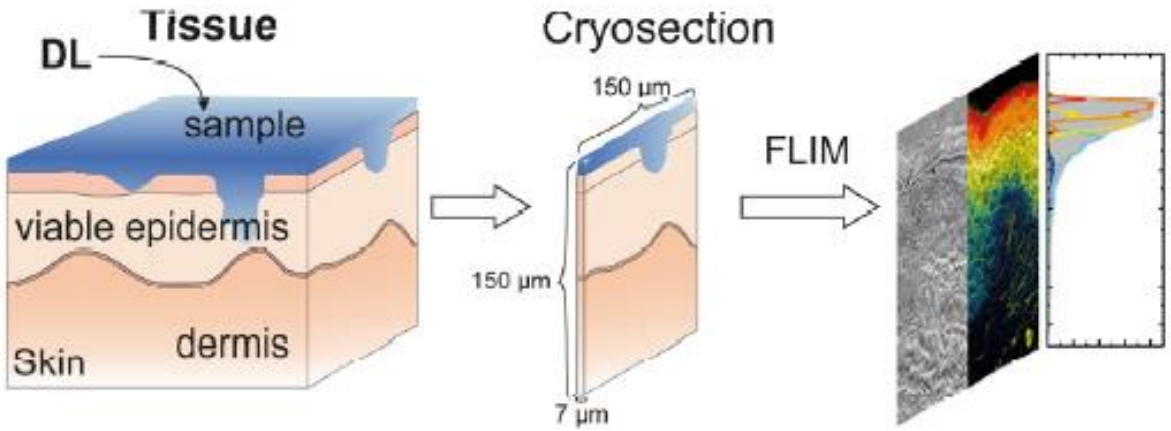


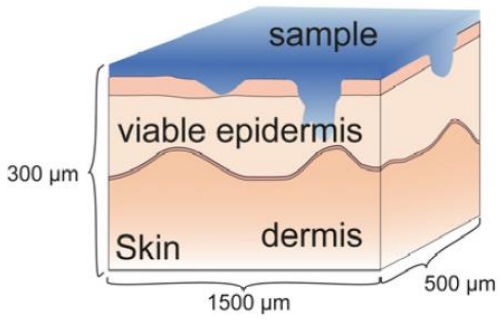
基于FLIM的可视化，利用DL荧光强度和寿命对组织中的分子浓度进行定量

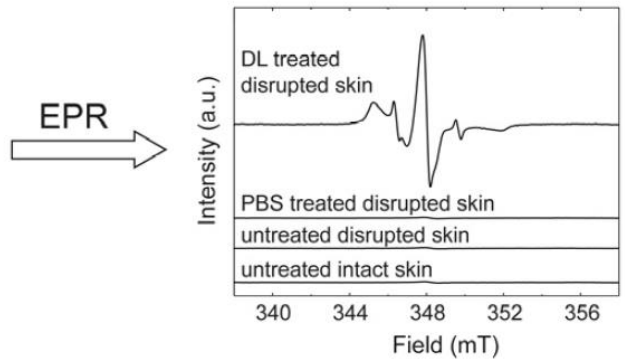
$$c_{DL}(I) = \frac{I_{ROI} / \#pixel\ in\ ROI \times image\ pixel}{\alpha} * \mathrm{MW} * F_r$$

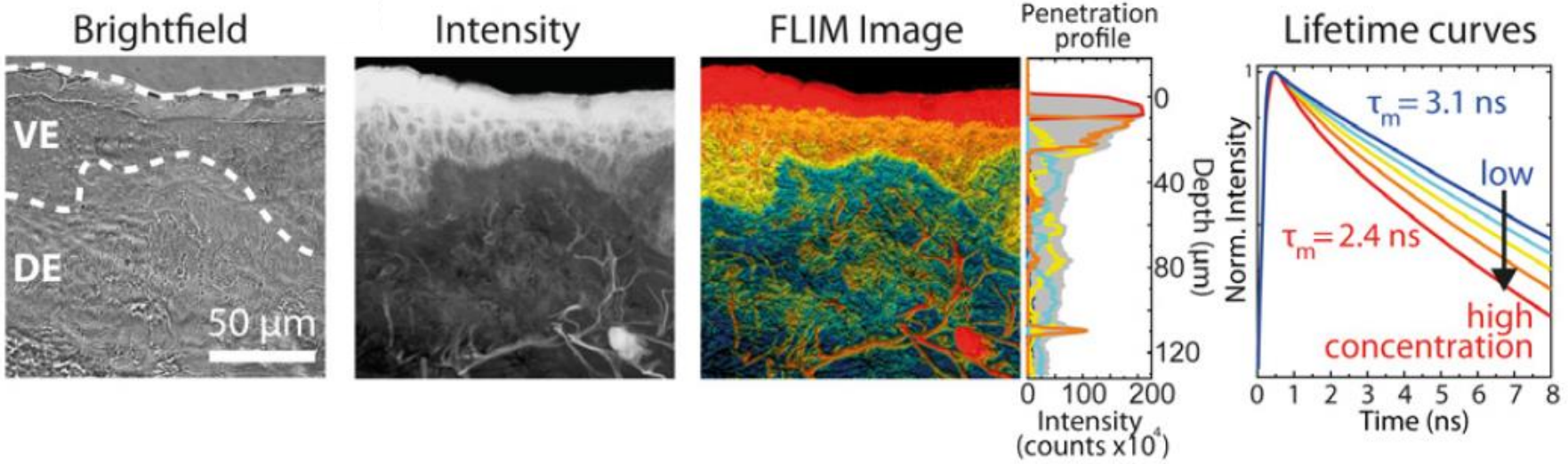
$$c_{DL}(\tau) = \sum_{cluster} \frac{\left(\frac{\tau_{cluster} - b}{\alpha}\right) * \#pixel_{cluster}}{total\ \#pixel\ in\ ROI} * MW * F_r$$

Experiments in human skin

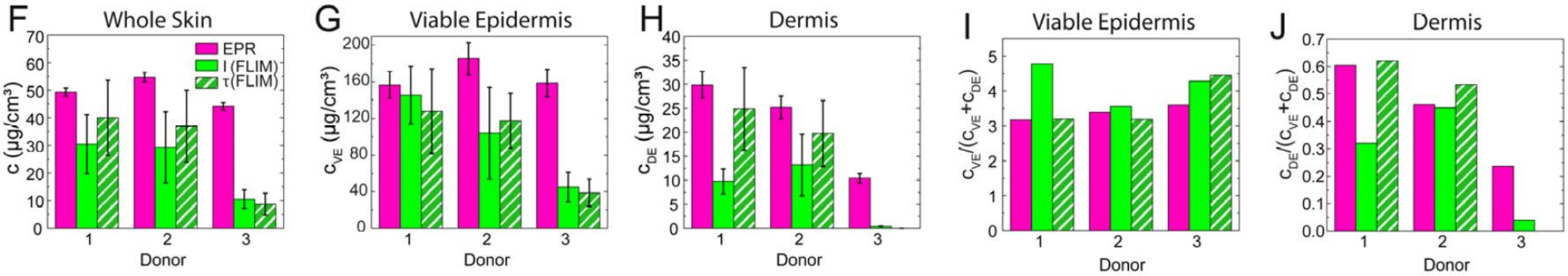








Experiments in human skin



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