

共聚焦激光显微内窥成像中 PMT 与 APD 探测器性能对比分析

侯冠廷¹, 徐宝腾^{1,2}, 周伟², 刘家林², 郭立泉², 杨西斌^{1,2*}

¹中国科学技术大学生物医学工程学院(苏州)生命科学与医学部, 江苏 苏州 215163;

²中国科学院苏州生物医学工程技术研究所光与健康研究中心, 江苏 苏州 215163

摘要 共聚焦激光显微内窥镜(CLE)可以进行在体组织显微成像,实现实时病理学诊断,而光电探测器作为 CLE 系统的核心部件,影响共聚焦图像质量。对比分析光电倍增管(PMT)和雪崩光电二极管(APD)在 CLE 中的性能表现,建立基于 PMT 和 APD 探测电路的 CLE 成像信号信噪比分析模型,理论分析光电探测器及其放大电路噪声、放大增益、输入光信号等参数对成像信号信噪比的影响。搭建基于 PMT 和 APD 双光路探测系统,对均匀荧光溶液和组织样品进行成像。研究结果表明,当样品荧光功率大于 10 nW 时,所选取 PMTSS 与 APD120A2 的输出图像质量接近,信噪比相差小于 0.67 dB,APD 以更高的性价比实现与 PMT 相当的成像性能。

关键词 医用光学; 共聚焦激光显微内窥镜; 荧光成像; 在体内窥; 雪崩光电二极管; 光电倍增管

中图分类号 TH773; TN152; TN364+.2

文献标志码 A

DOI: 10.3788/AOS222137

1 引言

共聚焦激光显微内窥镜(CLE)结合共聚焦显微成像技术和内窥镜成像技术,可以在体实时提供细胞分辨率的组织结构信息,从而提高消化道早期癌症的检出率。临床结果表明,其成像结果与活检病理图像呈现一致的细胞形态,有望实现在体“光学活检”^[1-5]。高效率的信号采集与传输有利于获取高质量的 CLE 图像,而光电探测器的选型是影响其成像质量的关键因素^[6-8]。法国 Mauna Kea Technologies 公司开发的 Cellvizio 系列产品中光电探测器选用雪崩光电二极管(APD);华中科技大学付玲课题组^[9-11]与中国科学院苏州生物医学工程技术研究所杨西斌课题组^[12-13]开发的 CLE 成像系统选用光电倍增管(PMT)。两种探测器的倍增机理有所不同:APD 利用外加电场下 PN 结内部的链式电离倍增初始光电流^[14-16],而 PMT 则利用真空管内部的多个打拿级在外加电场作用下倍增初始光电流^[17]。APD 具有高量子效率、低成本的优势;PMT 则具有高灵敏度、高增益的优势。

现有的 CLE 系统虽然选取不同类型的光电探测器,但选用更高扫描频率的共振振镜获得高帧率是 CLE 的重要发展方向,本课题组利用检流计式扫描振镜与 8 kHz 共振振镜的组合实现了 30 frame/s 帧率下

(512 pixel×512 pixel)的实时共聚焦内窥成像。高扫描频率的共振振镜带来图像帧率提升的同时,也缩短了荧光信号采集过程中的驻留时间^[18],降低了探测器探测的荧光强度,导致获取的 CLE 图像信噪比(SNR)降低。

本文针对研发的高帧率 CLE 成像系统,对比分析了 APD 和 PMT 选型对 CLE 图像信噪比的影响。首先,理论分析 APD 与 PMT 探测电路中噪声信号对 CLE 采集信号的影响,获得 CLE 信号的信噪比理论计算公式;其次,搭建双光路 CLE 成像系统,用于定量评估不同光电探测器选型(APD120A2 和 PMTSS)下 CLE 图像的信噪比。实验结果表明,APD 应用于 CLE 中可以达到与 PMT 极为相近的效果,当样品荧光强度高于 APD 的最低可探测光功率时,选用 APD 的性价比更高。本研究成果对 CLE 中探测器的选型具有指导意义。

2 实验系统与成像性能评估方法

2.1 CLE 实验装置及图像获取流程

图 1 为所搭建的 CLE 双光路成像系统。488 nm 激光器(Cobolt 06-MLD, HÜBNER Photonics)发出的激光由物镜(UPlanSApo 20×/0.75, Olympus)聚焦耦合到单模光纤中,通过准直透镜(F240FC-532,

收稿日期: 2022-12-13; 修回日期: 2023-01-08; 录用日期: 2023-02-09; 网络首发日期: 2023-02-20

基金项目: 国家重点研发计划(2021YFC2400100)、苏州市基础研究计划(szs2020308, SJC2022009)

通信作者: *yangxb@sibet.ac.cn

Thorlabs)后,经二向色镜(ZT488rdc, Chroma)、振镜模块(6215H&CRS 8 kHz, Cambridge Technology Inc.)、中继透镜组(AC254-050-A, AC254-100-A, Thorlabs)、耦合物镜(UPlanSApo 20×/0.75, Olympus)以及光纤探头后对样品进行扫描成像。激

发荧光经光纤束、二向色镜后的发射片(ET525/50m, Chroma)、小孔(P75D, Thorlabs),并通过50:50分束器(BSW10R, Thorlabs)分束后实现双光路同时成像。两条探测电路的输出信号同时被高速数据采集卡(QT1140VG-2CH, Queentest)采集。

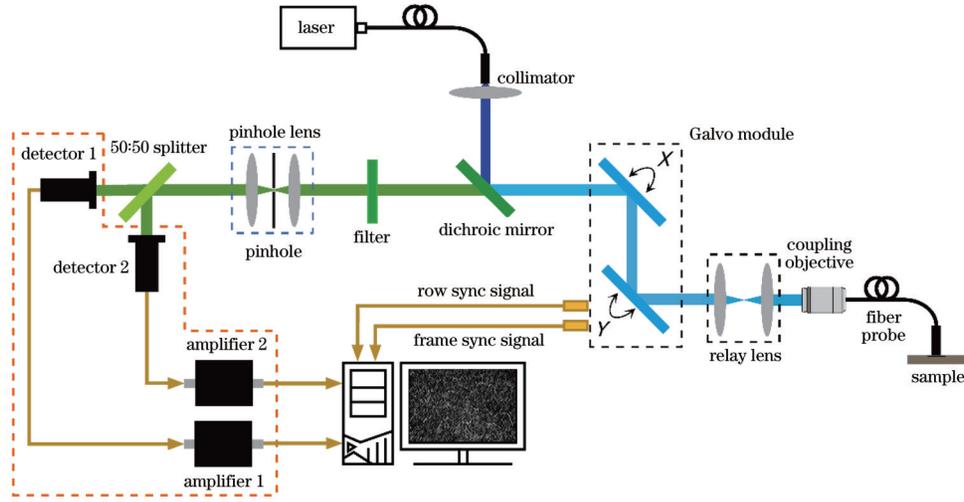


图 1 CLE 双光路成像系统实验装置图

Fig. 1 Schematic of dual optical path CLE system

CLE 图像的获取过程如图 2 所示。为了获取 CLE 原始图像[图 2(b)],需要对一维数据[图 2(a)]按照振镜模块发出的行同步、帧同步信号进行分割与

拼接。得到原始图像后,通过畸变与错位校正^[12-13, 19]得到最终用于图像信噪比分析的 CLE 图像[图 2(c)]。

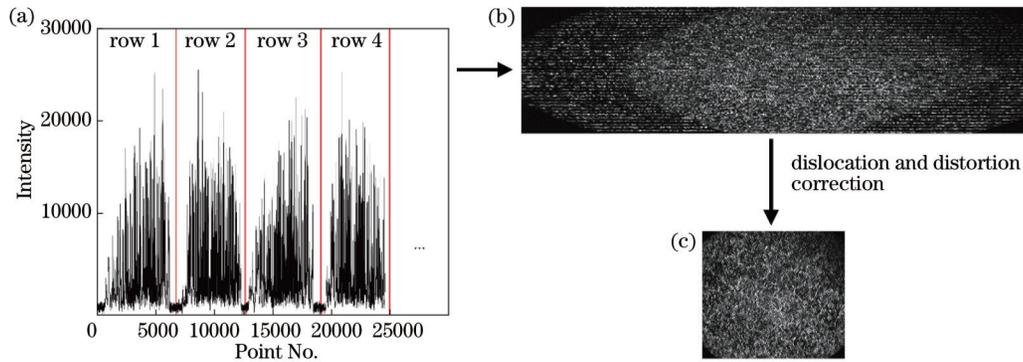


图 2 共聚焦图像的获取过程

Fig. 2 Acquisition process of confocal images

2.2 CLE 图像信噪比计算方法

基于互相关法校正 CLE 图像的错位与畸变问题^[13]。选取 150 pixel×150 pixel 的感兴趣区域(ROI)来计算图像信噪比。首先,利用高斯滤波方法(标准差为 2,滤波窗口为 9 pixel×9 pixel)对 ROI 图像进行处理,去除光纤束蜂窝状结构的影响;然后,利用局部最大值算法确定 ROI 中所有纤芯的中心位置;最后,基于纤芯中心位置的强度信息计算获得图像的信噪比。信噪比的计算流程如图 3 所示。

图像信噪比 R_{SN_Pic} 的计算方法^[20]为

$$R_{SN_Pic} = 20 \cdot \lg \left(\frac{I_{pic}}{I_f} \right), \quad (1)$$

$$I_{pic} = \frac{\sum_{i=1}^M \sum_{j=1}^N I_{ij}}{MN}, \quad (2)$$

$$I_f = \sqrt{\frac{\sum_{j=1}^M \sum_{i=1}^N (I_{ij} - \bar{I}_j)^2}{N \cdot (M - 1)}}, \quad (3)$$

$$\bar{I}_j = \frac{\sum_{i=1}^M I_{ij}}{M}, \quad (4)$$

式中: M 为用于计算图像信噪比的 ROI 图像总帧数; N 为 ROI 图像中提取出的纤芯总数; I_{ij} 为第 i 帧 ROI 图像中第 j 根纤芯中心位置的强度信息。

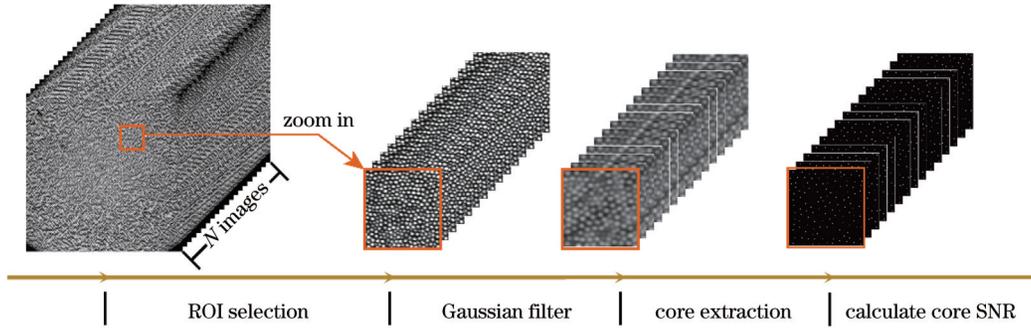


图 3 图像信噪比计算示意图

Fig. 3 Schematic of image signal-to-noise calculation

3 探测器选型及输出信噪比分析

3.1 探测器选型

选用两款光电探测器:PMTSS 和 APD120A2,各探测器参数见表 1。

3.2 基于 PMT 的 CLE 图像信噪比分析

由 2.2 节可知,CLE 图像信噪比的主要影响因素是采集信号的波动,而来自 PMT 或 APD 的散粒噪声和暗噪声,以及放大电路噪声等是信号波动的主要因素,本节将从理论分析噪声对 CLE 图像信噪比的影响。

基于 PMT 的 CLE 输出噪声 $V_{\text{outnoisePMT}}$ 表达式^[21]为

$$V_{\text{outnoisePMT}} = \sqrt{V_{\text{ShotPMT}}^2 + V_{\text{dPMT}}^2 + V_{\text{AmpPMT}}^2}, \quad (5)$$

式中: V_{ShotPMT} 为散粒噪声; V_{dPMT} 为暗噪声; V_{AmpPMT} 为放大电路噪声。散粒噪声与暗噪声的表达式^[17]为

$$V_{\text{ShotPMT}} = G_{\text{iv}} \cdot \sqrt{2eB\mu I_p}, \quad (6)$$

$$V_{\text{dPMT}} = G_{\text{iv}} \cdot \sqrt{2eB\mu I_d}, \quad (7)$$

式中: G_{iv} 为跨阻放大增益; e 为电子电荷; B 为系统带宽; μ 为 PMT 电流增益; I_p 为 PMT 阳极输出信号; I_d 为 PMT 阳极输出暗电流。

当入射光功率为 P 时, I_p 的表达式^[17]为

$$I_p = \frac{\mu \cdot \eta \cdot e \cdot P \cdot \lambda}{h \cdot c}. \quad (8)$$

CLE 输出信号 $V_{\text{outputPMT}}$ 的表达式为

$$V_{\text{outputPMT}} = G_{\text{iv}} \cdot I_p, \quad (9)$$

式中: η 为 PMT 的量子效率; λ 为入射光波长; h 为普朗

克常数; c 为光速。

结合式(1)与式(5)~(9),基于 PMT 的 CLE 图像信噪比 $R_{\text{SN,PMT}}$ 的表达式为

$$R_{\text{SN,PMT}} = 20 \cdot \lg \left[\frac{\frac{\eta \cdot e \cdot P \cdot \lambda}{h \cdot c}}{\sqrt{2e \cdot B \left(\frac{\eta \cdot e \cdot P \cdot \lambda}{h \cdot c} + \frac{I_d}{\mu} \right) + \frac{V_{\text{AmpPMT}}^2}{\mu^2 \cdot G_{\text{iv}}^2}}} \right]. \quad (10)$$

3.3 基于 APD 的 CLE 图像信噪比分析

基于 APD 的 CLE 输出噪声 $V_{\text{outnoiseAPD}}$ 表达式为

$$V_{\text{outnoiseAPD}} = \sqrt{V_{\text{ShotAPD}}^2 + V_{\text{dAPD}}^2 + V_{\text{AmpAPD}}^2}, \quad (11)$$

式中: V_{ShotAPD} 为散粒噪声; V_{dAPD} 为暗噪声; V_{AmpAPD} 为放大电路噪声。散粒噪声与暗噪声的表达式^[22]为

$$V_{\text{ShotAPD}} = G_v \cdot \sqrt{2eBM_a I_L F}, \quad (12)$$

$$V_{\text{dAPD}} = G_v \cdot \sqrt{2eBM_a I_{\text{dAPD}} F}, \quad (13)$$

式中: G_v 为电压放大增益; M_a 为 APD 雪崩增益; F 为倍增噪声因子; I_L 为 APD 输出信号^[23]; I_{dAPD} 为 APD 输出暗电流。

CLE 输出信号 $V_{\text{outputAPD}}$ 的表达式为

$$V_{\text{outputAPD}} = G_v \cdot I_L, \quad (14)$$

$$I_L = \frac{M_a \cdot \eta \cdot e \cdot P \cdot \lambda}{h \cdot c}. \quad (15)$$

结合式(1)与式(11)~(15),基于 APD 的 CLE 图像信噪比 $R_{\text{SN,APD}}$ 的表达式为

表 1 探测器的配置和参数

Table 1 Configuration and parameters of detectors

Parameter	PMT	APD
Detector	PMTSS	APD120A2
Price (CNY)	28663.91	11944.83
Active area diameter	3.7 mm × 13.0 mm	Φ1 mm
Bandwidth /MHz	250	50
Dark current /nA	2(Typical);10(Max)	—
NEP /($\mu\text{W} \cdot \text{Hz}^{-1/2}$)	—	0.21 (DC-50 MHz)
Amplifier	C9999(50 mV/ μA)	DHPVA-101(20 dB)

$$R_{SN_APD} = 20 \cdot$$

$$\lg \left[\frac{M_a \cdot \eta \cdot e \cdot P \cdot \lambda}{h \cdot c} \right] \cdot \left[\sqrt{2e \cdot B \left(M_a \cdot \frac{M_a \cdot \eta \cdot e \cdot P \cdot \lambda}{h \cdot c} + I_{dAPD} \right) F + \frac{V_{AmpAPD}^2}{G_v^2}} \right] \quad (16)$$

由于使用 APD 模块,利用其手册中的响应度 R_s 、模块跨阻增益 G_{module} 以及噪声等效功率 $P_{NE}^{[24-25]}$,式(16)可进一步表示为

$$R_{SN_APD} = 20 \cdot$$

$$\lg \left[\frac{PR_s G_{module}}{\sqrt{2eBM_a PR_s G_{module} F + P_{NE}^2 BR_s^2 + \frac{V_{AmpAPD}^2}{G_v^2}}} \right] \quad (17)$$

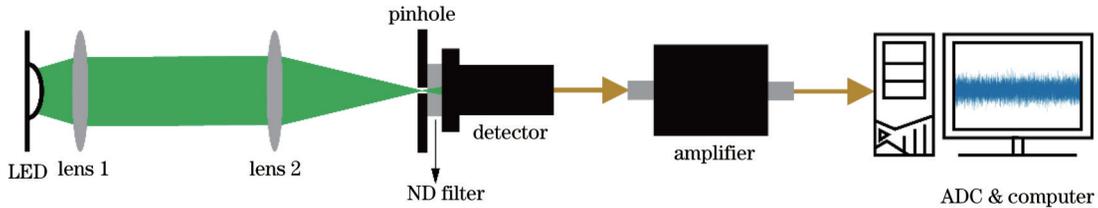


图 4 探测器性能对比实验装置图

Fig. 4 Detector performance comparison experiment device

4.2 探测器性能对比测试结果

图 5 给出了不同增益电压和光功率下 PMTSS 输出信号信噪比 R_{SN_PMT} 的理论和实验结果,其中散点为实验结果,各曲线为根据式(10)得到的理论计算结果,理论计算所使用的参数如表 3 所示。由图 5 可知, R_{SN_PMT} 随着光功率 P 和增益的增加而增大,通过改变增益来提高 R_{SN_PMT} 存在上限, R_{SN_PMT} 理论最大值为 32 dB,实验中最大值为 33 dB,理论与实验结果吻合,相对误差小于 4.8%。

图 6(a)所示为不同光功率下 APD120A2 输出信

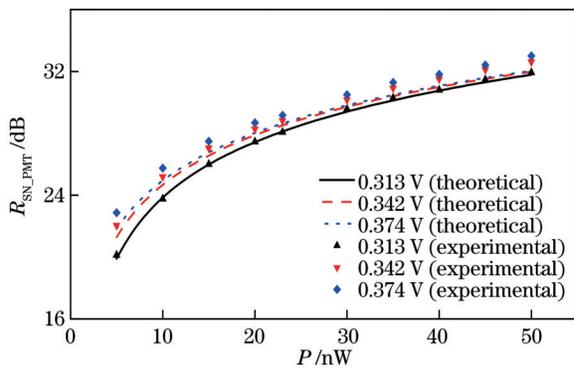


图 5 PMTSS 的输出信噪比随光功率的变化

Fig. 5 R_{SN_PMT} of PMTSS varying with optical power

4 探测器性能对比测试

4.1 实验系统

探测器性能对比实验系统如图 4 所示。525 nm 波长的 LED 光经准直、聚焦后穿过小孔 (P150D, Thorlabs),经过中性衰减片 (ZAB5, PHTODE) 后被光电探测器接收,光电探测器产生的电信号经过放大、A/D 转换后,传输到上位机中进行处理。具体实验数据采集条件见表 2。

表 2 探测器性能对比实验的数据采集条件

Table 2 Data collection conditions of detector performance comparison experiment

Condition	PMT	APD
Detector	PMTSS	APD120A2
Optical power	Start at 5 nW, end at 50 nW, step size is 5 nW	
Sample rate	125 MHz	
Gain voltage	0.313 V, 0.342 V, 0.374 V	fixed

表 3 信噪比计算中使用的参数数值

Table 3 Parameter values used for the SNR calculation

Parameter	Value	Parameter	Value
η	24% (PMTSS)	V_{AmpPMT}	2.6 mV (RMS)
e	1.6×10^{-19} C	V_{AmpAPD}	283 μ V (RMS)
λ	525 nm	P_{NE}	0.21 pW/ $\sqrt{\text{Hz}}$
h	$6.62607015 \times 10^{-34}$ J·s	G_{iv}	50000
c	3×10^8 m/s	G_{module}	50000
B	10 MHz	G_v	10
I_d	10 nA	R_s	22.3 A/W
M_a	50	F	$M_a^{0.4}$

号信噪比 R_{SN_APD} 的理论和实验结果,其中散点为实验结果,曲线为由式(17)得到的理论计算结果,理论计算所使用的参数如表 3 所示。由图 6(a)可知, R_{SN_APD} 随着光功率 P 的增加而增大, R_{SN_APD} 理论最大值为 30.64 dB,实验中最大值为 31 dB,理论与实验结果吻合得较好,相对误差小于 1.67%。

图 6(b)所示为不同光功率下 APD120A2 和 $V_{Gain}=0.342$ V 时 PMTSS 的输出信号信噪比实验结果。由图 6(b)可知,该条件下 APD120A2 与 PMTSS 的输出信噪比实验结果平均相差 2.74 dB。

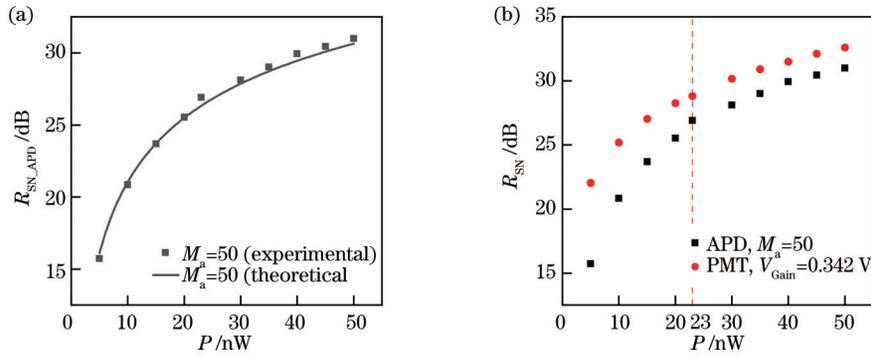


图 6 探测器性能对比。(a)APD120A2的输出信噪比随光功率的变化;(b)不同探测器的输出信噪比随光功率的变化
Fig. 6 Comparison of detector performance. (a) $R_{SN,APD}$ of APD120A2 varying with optical power; (b) R_{SN} of different detectors varying with optical power

5 CLE 成像实验

5.1 均匀荧光溶液实验及结果

本实验使用图 1 所示的共聚焦激光显微内窥双光路成像系统,该系统通过设置 50:50 分束器使得探

测器前成像系统的参数一致,保证了使用不同探测器的 CLE 系统实验结果具有可比性。实验前,确保物镜焦点在光纤端面上。实验需采集不同质量浓度的均匀荧光溶液的共聚焦图像,具体实验条件如表 4 所示。

表 4 均匀荧光溶液实验条件

Table 4 Uniform fluorescent solution experimental conditions

Condition	PMT	APD
Detector	PMTSS	APD120A2
Solution	75 mL fluorescein sodium solution with mass concentration of 0.0067%, 0.0133%, 0.02%, 0.0267%, and 0.0333% g/mL	
Gain voltage	0.342 V	fixed
Sample rate	125 MHz	
Amount of data	Acquire 120 consecutive images for each condition	

图 7 所示为不同光功率下基于 APD120A2 和 PMTSS 的 CLE 图像信噪比 $R_{SN,Pic}$ 的实验结果。由图 7 可知, $R_{SN,Pic}$ 随着溶液质量浓度的增加而增大,且

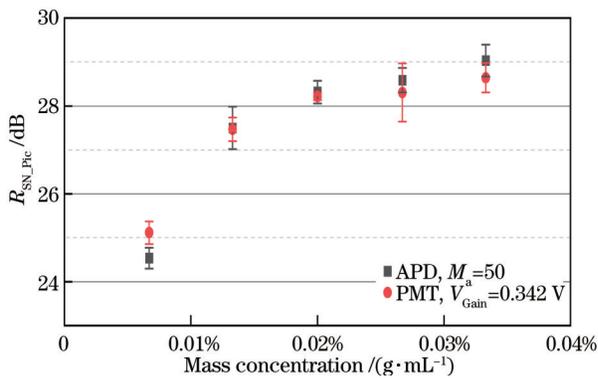


图 7 APD120A2 和 PMTSS 的均匀荧光溶液成像实验结果
Fig. 7 Experimental results of uniform fluorescent solution imaging of APD120A2 and PMTSS

APD120A2 的 $R_{SN,Pic}$ 与 $V_{Gain}=0.342$ V 时 PMTSS 的 $R_{SN,Pic}$ 几乎相等。实验结果表明,CLE 工作中由于受到荧光淬灭等因素的影响,APD120A2 与 PMTSS 的成像性能差距缩小。

5.2 荧光样品实验及结果

荧光样品实验也使用图 1 所示的共聚焦激光显微内窥双光路成像系统,实验中显微成像系统参数与均匀荧光溶液实验中的参数相同。在荧光样品实验中, PMTSS 的增益电压为 0.342 V。实验过程中将各荧光样品固定在载玻片上,通过三维位移台移动载玻片对各样品的不同部位进行成像,样品选取及制备方法见表 5。针对任意部位共聚焦图像,本实验选取 25 个无重叠的 ROI,利用 2.2 节方法分别计算出各 ROI 的图像信噪比,以其均值为该区域图像的信噪比。

图 8 第一、二列给出了处理后的 APD120A2 和 PMTSS 探测的样品图像,第三列给出了由原始图像数据统计得到的不同光功率的像素个数分布曲线。光功率由像素强度值转化为电压值后通过式(8)~(9)、(14)~(15)计算得到。图 8(a)、(b)所示样品为 13 μ m 直径的荧光小球(S4),图像中小球排布整齐,形状规则;图 8(d)、(e)所示样品为擦镜纸(S3),图像中纤维相互堆叠、清晰可见,表现出 CLE 较强的光学层析成像能力;图 8(g)、(h)所示样品为叶片上表皮(S2),图像中细胞壁棱角分明;图 8(j)、(k)所示样品为叶片下表皮

(S1), 图像中气孔及腰果状保卫细胞结构清晰可见。

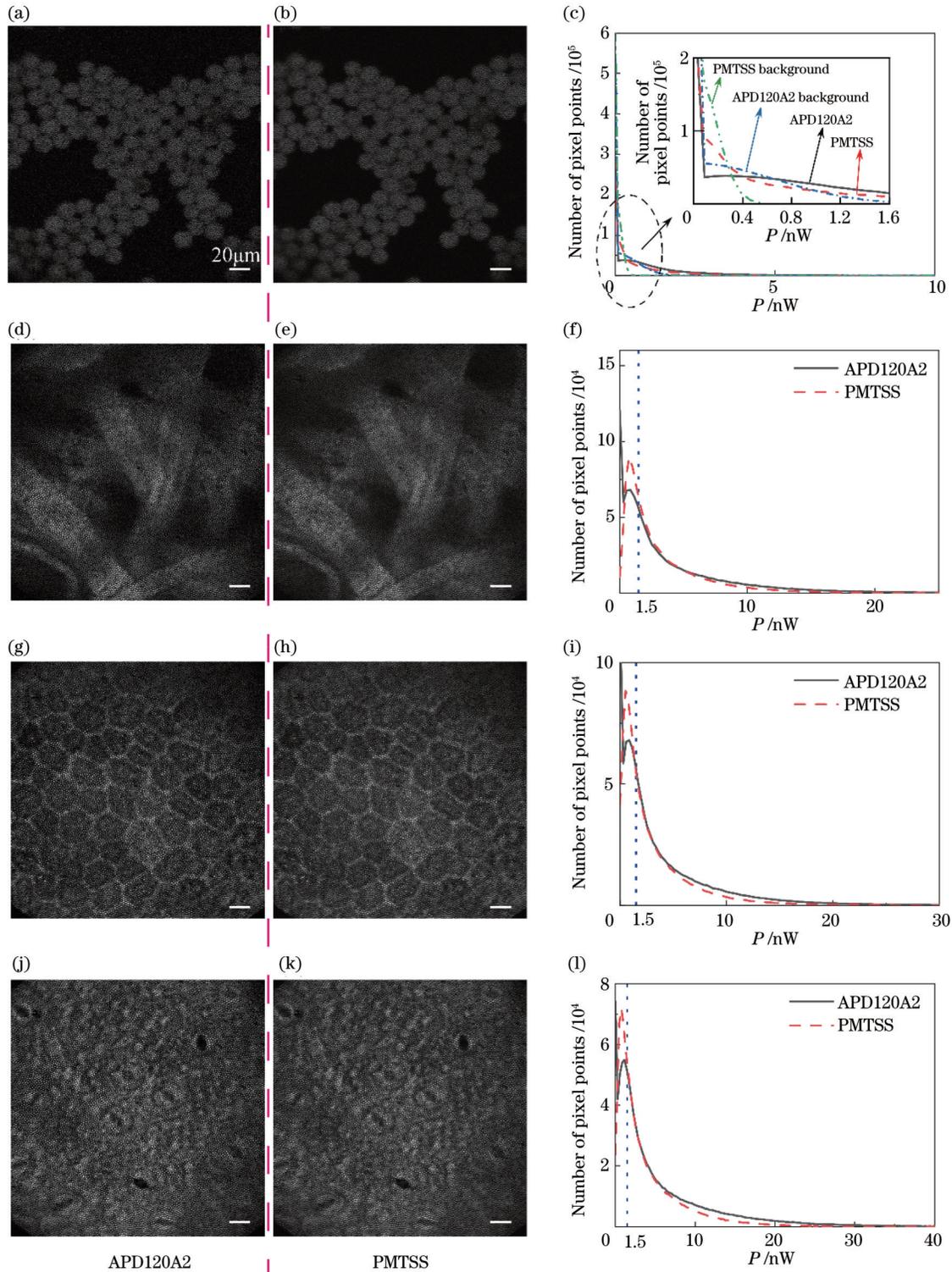


图 8 荧光样品成像结果及不同光功率下像素点个数统计结果。(a)~(c) 荧光小球; (d)~(f) 擦镜纸; (g)~(i) 石楠叶片上表皮; (j)~(l) 石楠叶片下表皮

Fig. 8 Imaging results of fluorescent samples and statistics results of the number of pixel points under different optical powers. (a)~(c) Fluorescent beads; (d)~(f) lens tissue; (g)~(i) upper epidermis of *Photinia serrulata* leaves; (j)~(l) lower epidermis of *Photinia serrulata* leaves

图 8(c) 所示为暗背景图像和小球图像的像素分布, 可以看到, 基于 PMTSS 的 CLE 系统暗背景像素集中在 $0 \sim 0.6 \text{ nW}$ 的光功率范围, 而基于 APD120A2 的

CLE 系统暗背景像素分布在 $0 \sim 1.6 \text{ nW}$ 的范围。由上述结果可知, APD120A2 系统的暗背景图亮度波动较大、暗噪声较高, 这将明显影响 CLE 系统在弱光成像

表 5 荧光样品选取及制备

Table 5 Fluorescence sample selection and preparation

No.	Sample	Preparation
S1	Lower epidermis of <i>Photinia serrulata</i> leaves	Avoid the main veins and cut out a number of small square leaves, soak them in 1% sodium fluorescein solution for 96 h, rinse and dry the epidermis before the experiment
S2	Upper epidermis of <i>Photinia serrulata</i> leaves	Avoid the main veins and cut out a number of small square leaves, soak them in 1% sodium fluorescein solution for 72 h, rinse and dry the epidermis before the experiment
S3	Lens tissue	0.0333% sodium fluorescein solution sprayed and dried
S4	13 μm fluorescent beads	Pipette 5 μL of fluorescent beads(FL-PS-G-13, TJDAE technology) onto the glass slide and dry

时的图像信噪比。

图 9(a)给出了本实验中各样品不同部位实验图像的 $R_{SN,Pic}$ 统计结果;图 9(b)给出了不同平均荧光功率(P_{avg})下各样品的 $R_{SN,Pic}$ 实验结果,其中 P_{avg} 为将 I_{pic} 代入式(9)与式(14)计算得出。由图 9(a)可知,S1、S2 样品的 APD120A2 与 PMTSS 成像效果十分接近,S3、S4 样品的 APD120A2 成像效果不如 PMTSS。由图 9(b)可知,APD120A2 与 PMTSS 的图像信噪比差距随

P_{avg} 的增大而降低,最终趋于稳定。该实验结果表明:当 $P_{avg} < 10$ nW,基于 APD120A2 的 CLE 系统图像信噪比低于基于 PMTSS 的 CLE 系统,结合前文对图 8(c)的分析,说明在 $P_{avg} < 10$ nW 时,暗噪声是系统图像信噪比的主要影响因素;当 $P_{avg} > 10$ nW,基于 APD120A2 的 CLE 系统图像信噪比与基于 PMTSS 的 CLE 系统相当。

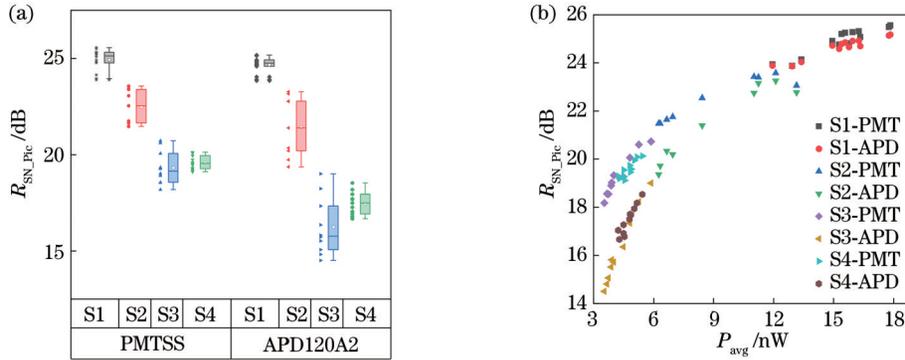


图 9 荧光样品成像的图像信噪比汇总。(a)图像信噪比的箱线图;(b)图像信噪比与成像平均光功率的关系

Fig. 9 Summary of $R_{SN,Pic}$ for imaging fluorescent samples. (a) Boxplot of image SNR; (b) relationship between image SNR and imaging average optical power

6 结 论

详细研究对比了光电探测器选型差异对 CLE 系统成像性能的影响。首先,对由不同探测器与级联放大器构成的探测电路输出信号信噪比进行理论建模;其次,为评估 APD 和 PMT 应用于 CLE 的实际效果,搭建了双光路 CLE 实验系统。实验结果表明,在平均荧光功率高于 10 nW 时,基于 APD120A2 的 CLE 的成像性能可与基于 PMTSS 的 CLE 媲美。因此在 CLE 设计中,确定 CLE 要探测的光功率范围,视情况选择 APD 或 PMT 作为光电探测器,可得到更具性价比的成像结果。

本实验中基于 APD120A2 的 CLE 成像性能未超过基于 PMTSS 的 CLE。未来将基于所设计的信噪比模型,通过选择倍增噪声因子更低的 APD 并自主设计低噪声 APD 外围电路,进一步提高基于 APD 的 CLE 系统成像质量。

参 考 文 献

- [1] Kim B, Kim Y H, Park S J, et al. Probe-based confocal laser endomicroscopy for evaluating the submucosal invasion of colorectal neoplasms[J]. Surgical Endoscopy, 2017, 31(2): 594-601.
- [2] Neumann H, Kiesslich R, Wallace M B, et al. Confocal laser endomicroscopy: technical advances and clinical applications[J]. Gastroenterology, 2010, 139(2): 388-392.
- [3] Aisenberg J. Gastrointestinal endoscopy nears the molecular era [J]. Gastrointestinal Endoscopy, 2008, 68(3): 528-530.
- [4] Kiesslich R, Burg J, Vieth M, et al. Confocal laser endoscopy for diagnosing intraepithelial neoplasias and colorectal cancer *in vivo*[J]. Gastroenterology, 2004, 127(3): 706-713.
- [5] 杨雪芳, 刘哲晰, 王璞. 共聚焦内窥镜显微成像技术及其应用[J]. 中国激光, 2022, 49(19): 1907002. Yang X F, Liu Z X, Wang P. Confocal endoscopic microscopy and its applications[J]. Chinese Journal of Lasers, 2022, 49(19): 1907002.
- [6] Sheppard C J R, Gan X S, Gu M, et al. Signal-to-noise ratio in confocal microscopes[M]//Pawley J B. Handbook of biological confocal microscopy. Boston: Springer, 2006: 442-452.
- [7] Pawley J B. Fundamental limits in confocal microscopy[M]//

- Pawley J B. Handbook of biological confocal microscopy. Boston: Springer, 2006: 20-42.
- [8] Pawley J B. Points, pixels, and gray levels: digitizing image data [M]//Pawley J B. Handbook of biological confocal microscopy. Boston: Springer, 2006: 59-79.
- [9] 张红明. 基于光纤束的共聚焦荧光内窥镜成像研究[D]. 武汉: 华中科技大学, 2013: 14-16.
Zhang H M. Research on fiber bundle confocal fluorescence microendoscopy[D]. Wuhan: Huazhong University of Science and Technology, 2013: 14-16.
- [10] 许瑞. 医用近红外共聚焦内窥镜研究[D]. 武汉: 华中科技大学, 2019: 41-44.
Xu R. Study of medical near-infrared confocal endoscopy[D]. Wuhan: Huazhong University of Science and Technology, 2019: 41-44.
- [11] Wang J F, Yang M, Yang L, et al. A confocal endoscope for cellular imaging[J]. Engineering, 2015, 1(3): 351-360.
- [12] 徐宝腾, 杨西斌, 刘家林, 等. 高速扫描激光共聚焦显微内窥镜图像校正[J]. 光学精密工程, 2020, 28(1): 60-68.
Xu B T, Yang X B, Liu J L, et al. Image correction for high speed scanning confocal laser endomicroscopy[J]. Optics and Precision Engineering, 2020, 28(1): 60-68.
- [13] Xu B T, Yang X B, Liu J L, et al. Real-time correction of transverse dislocation and distortion in probe-based confocal laser endomicroscopy imaging[J]. Optics Communications, 2022, 522: 128653.
- [14] 徐衬村. 雪崩光电二极管的边击穿抑制及过剩噪声因子测试[D]. 武汉: 华中科技大学, 2015: 3-14.
Xu C C. The study on suppression of edge pre-breakdown and excess noise factor measurement of APDs[D]. Wuhan: Huazhong University of Science and Technology, 2015: 3-14.
- [15] 崔星宇, 林逢源, 张志宏, 等. 低噪声 InGaAs/InP 雪崩光电二极管的模拟分析[J]. 中国激光, 2021, 48(17): 1701001.
Cui X Y, Lin F Y, Zhang Z H, et al. Simulation analysis of low-noise InGaAs/InP avalanche photodiodes[J]. Chinese Journal of Lasers, 2021, 48(17): 1701001.
- [16] 张维宇, 汪洋, 金湘亮. 双波峰响应 CMOS 单光子探测器的设计与优化[J]. 光学学报, 2021, 41(17): 1704001.
Zhang W Y, Wang Y, Jin X L. Design and optimization of double-wave-peak response CMOS single-photon detector[J]. Acta Optica Sinica, 2021, 41(17): 1704001.
- [17] Hamamatsu photonics K. K. Editorial Committee. Photomultiplier tubes: basics and applications[M]. 4th ed. Hamamatsu: Hamamatsu Photonics K. K. Electron Tube Division, 2017: 14-20.
- [18] Zhou A N, Engelmann S A, Mihelic S A, et al. Evaluation of resonant scanning as a high-speed imaging technique for two-photon imaging of cortical vasculature[J]. Biomedical Optics Express, 2022, 13(3): 1374-1385.
- [19] Huang F, Qian Z H, Fang C T, et al. Pixel dislocation correction method for a laser confocal scanning microscope through the nonlinear triangular wave driving mode and square wave index reconstruction[J]. Applied Optics, 2021, 60(12): 3492-3500.
- [20] 国家食品药品监督管理总局. 医用内窥镜内窥镜功能供给装置摄像系统: YY/T 1603—2018[S]. 北京: 中国标准出版社, 2018.
National Medical Products Administration. Medical endoscopes: endoscope supply units: video camera system: YY/T 1603—2018[S]. Beijing: Standards Press of China, 2018.
- [21] 李乐, 汪龙祺, 黄煜, 等. 光电探测系统噪声特性研究与降噪设计[J]. 光学精密工程, 2020, 28(12): 2674-2683.
Li Y, Wang L Q, Huang Y, et al. Study on noise characteristics of photoelectric detection system and noise reduction design[J]. Optics and Precision Engineering, 2020, 28(12): 2674-2683.
- [22] Hamamatsu photonics K. K. Solid State Division. Si APD technical note[EB/OL]. (2021-10-05) [2022-11-16]. <https://www.hamamatsu.com>.
- [23] 刘贺雄, 周冰, 高宇辰. APD 探测系统的噪声特性及其影响因素研究[J]. 激光技术, 2018, 42(6): 862-867.
Liu H X, Zhou B, Gao Y C. Research of noise characteristics and influence factors of APD detection systems[J]. Laser Technology, 2018, 42(6): 862-867.
- [24] Mackowiak V, Peupelmann J, Ma Y, et al. NEP-noise equivalent power[EB/OL]. (2015-07-31)[2022-11-16]. <https://www.thorlabs.com>.
- [25] Thorlabs. APD110x/120x operation manual[EB/OL]. (2019-12-30)[2022-11-16]. <https://www.thorlabs.com>.

Comparative Analysis of the Performance of PMT and APD Detectors in Confocal Laser Endomicroscopy

Hou Guanting¹, Xu Baoteng^{1,2}, Zhou Wei², Liu Jialin², Guo Liquan², Yang Xibin^{1,2*}

¹*Division of Life Sciences and Medicine, School of Biomedical Engineering (Suzhou), University of Science and Technology of China, Suzhou 215163, Jiangsu, China;*

²*Center of Light and Health, Suzhou Institute of Biomedical Engineering and Technology, Chinese Academy of Sciences, Suzhou 215163, Jiangsu, China*

Abstract

Objective Confocal laser endomicroscopy (CLE) is an emerging imaging method with a cellular resolution for obtaining histopathological images of structure information on the mucosa in real time. CLE can significantly improve the detection rate of early tumors. Usually, the fluorescence from the tissue pre-stained with exogenous fluorescent dyes can be detected by the photodetector, which further generates corresponding electrical signals by the photoelectric effect and internal multiplication. Then, the electrical signal can be amplified to form CLE images after analog-to-digital conversion. Meanwhile, the output signal of the photodetector is influenced by the noise, which will further affect the quality of the final image. The noise in CLE mostly includes the shot noise introduced by the inherent fluctuation of obtained photons

and the dark noise of detectors. So far, many researchers have mainly proposed CLE using mainly two types of photodetectors, such as avalanche photodiode (APD) and photomultiplier tube (PMT). Usually, APD has the advantage of high quantum efficiency and low cost. PMT has the advantage of high gain and low noise. Therefore, the advantages of the selected photodetectors are very different. At present, no researcher has thoroughly investigated the performance differences between APD and PMT. This paper presents a detailed comparative study of APD and PMT performance in CLE.

Methods Based on the different working principles of APD and PMT, the quantitative model for output signal-to-noise ratio (SNR) in CLE is obtained. The parameters include optical power, quantum efficiency and internal gain of the detector, system bandwidth, photocurrent, dark current, and amplifier noise. All parameters can be obtained from the user manual. Then, we implement a detector performance comparison test, and the light emitted from the LED of 525 nm is used to simulate the fluorescence of samples after passing through a neutral density filter and pinhole. This test not only provides a preliminary understanding of the differences between APD120A2 and PMTSS but also verifies the validity of the proposed model. Eventually, the most important CLE imaging experiment is carried out. For this experiment, a dual optical path CLE imaging system is established which allows simultaneous imaging with APD120A2 and PMTSS under the same optical system by using the beam splitter of 50:50. The fluorescent samples for this experiment are fluorescein sodium solution, fluorescent beads with diameter of 13 μm , lens tissue (stained by sodium fluorescein), and *Photinia serrulata* leaves (stained by sodium fluorescein). Distribution of the number of pixels with different optical powers and image SNR is the quantitative evaluation index of the images in this experiment.

Results and Discussions Validity of the detector output SNR model for CLE is verified by the detector performance comparison test. This test shows that the SNR of APD120A2 is 2.74 dB on average lower than that of PMTSS. The theoretical value of the model matches with the experimental results, and the correlation error is less than 4.8% (Fig. 5 and Fig. 6). In addition, there is an upper limit to increase the SNR of PMTSS by adding the internal gain of PMT. By imaging the fluorescein sodium solution, we find that due to fluorescence quenching and other factors, the image SNR difference between APD120A2 and PMTSS is 0.28 dB on average (Fig. 7), which is much smaller than the result in detector performance comparison test. The imaging results of other fluorescence samples indicate that the dark current of APD120A2 is higher than that of PMTSS, which affects the low light detection capability of APD120A2. When the average fluorescence optical power is less than 10 nW, the difference in image SNR between APD120A2-based CLE and PMTSS-based CLE increases with decreasing optical power. APD120A2-based CLE imaging performance is comparable to PMTSS-based CLE imaging performance when the average fluorescence optical power is greater than 10 nW. Under this condition, the difference of image SNR is less than 0.67 dB (Fig. 9). Thus, when the optical power is lower than 10 nW, APD120A2 is not recommended for CLE because of the higher dark noise.

Conclusions This paper presents a detailed study to compare the influence of the selected differences in photodetector on the imaging performance of CLE systems. Firstly, a quantitative model for APD-based and PMT-based CLE SNR is proposed considering different parameters. Secondly, a comparison test for detector performance is carried out to verify the model, which aims to understand the characteristics of APD and PMT. In the end, a dual optical path CLE experimental system is built to evaluate the practical effects of APD120A2 and PMTSS in CLE. The experimental results show that the imaging performance of the APD120A2-based CLE is comparable to that of the PMTSS-based CLE when the average fluorescence optical power is higher than 10 nW. Therefore, in the design of the CLE, it is necessary to determine the detected optical power range for CLE. Selecting APD instead of PMT as the CLE photodetector can obtain more cost-effective imaging results. In the future, based on the SNR model in this paper, we will further improve the imaging quality of APD-based CLE systems by selecting APDs with lower noise factors and designing low-noise APD peripheral circuits. The research results can also provide guidance for selecting photodetectors in low-light detection applications.

Key words medical optics; confocal laser endomicroscopy; fluorescence imaging; *in vivo* endoscopy; avalanche photodiode; photomultiplier tube