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基于单相机的微米谱域偏振光学相干层析成像方法

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摘要:提出一种基于单相机的偏振谱域光学相干层析方法。该方法基于全单模光纤光路,使用宽带光源实现微米纵向高分辨率成像性能。通过硬件和软件两方面对系统色散进行矫正补偿,提高系统的实际分辨率。为实现样本偏振态的测量,入射至样品表面及参考臂的光偏振态由偏振片和四个偏振控制器进行调制。此外,利用偏振片在不同时间的通道切换实现两正交通道光谱信号的分时探测。根据得到的正交光谱信息,计算重构样品强度图和相位延迟图。该系统成功实现了单相机的偏振 OCT 测量,通过获取离体生物组织的强度图和相位延迟图验证了系统的成像能力。该方法为实现小型化在体高分辨偏振成像打下基础。

关键词:生物医学成像;偏振敏感光学相干层析;单相机探测;色散补偿

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0 引言

光学相干层析(Optical Coherence Tomography, OCT)技术是一种能够对薄层组织进行层析成像的医学成像技术,具有无创、高分辨等特点,成像深度达到毫米量级,纵向分辨率可至 $1\ \mu\text{m}$ ^[1]。谱域光学相干层析(Spectral Domain Optical Coherence Tomography, SDOCT)是 OCT 技术分类中的一种,相较于时域 OCT, SDOCT 参考臂中无需添加扫描机构,具有更快的成像速度、更高的信噪比和灵敏度^[2]。OCT 已被广泛应用于眼科^[3]、皮肤科^[4]等医学检测领域,同时该技术也被应用于材料科学领域^[5-6]。

偏振敏感光学相干层析成像技术(Polarization Sensitive Optical Coherence Tomography, PSOCT)是 OCT 技术的功能扩展,它在光学相干层析技术的基础上添加了测量并分析样品偏振信息的功能。其不仅能够反应样品内部的微观结构信息,还能够探测偏振光入射到样品后不同深度偏振态的改变情况,采用相关偏振矩阵计算表征偏振特性,如相位延迟、光轴方向等,从而可以辨别普通 OCT 无法区分的特性结构^[7-8]。PSOCT 技术已被用于检测皮肤烧伤程度^[9]、牙齿修复检测^[10]、人体肺部肿瘤评估^[11]以及视网膜病变检测^[12]等医学领域,具有重要的应用价值。

一般的 OCT 系统轴向分辨率在 $3\ \mu\text{m}$ 以上,为了能够看清组织更细微的结构,需要实现更高的分辨率功能。SDOCT 系统轴向分辨率与光源的带宽和中心波长有关,为了有效提高系统分辨率,需要使用中心波长较短或者带宽更宽的光源,但由于生物光学窗口的限制,常用方法即增加成像的光源带宽。具体做法是采用光谱拼接^[13]和使用超连续谱激光器(Super-continuum Light Source, SCL)^[14],随着分辨率的提高,能够实现活体组织亚细胞成像^[15-16]等功能。

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随着光源带宽的增加,系统引入色散,干涉包络信号展宽,导致成像模糊,分辨率降低。通常基于硬件和软件两方面的补偿解决色散。硬件补偿通常是指在系统参考臂中加入两臂色散差的等效介质。例如可变厚度熔融硅和BK7棱镜^[17]、一定厚度的水槽^[18]、不同类型长度的光纤^[19]等。基于硬件的还可以使用快速扫描延迟线系统^[20]。软件方面提出了多种补偿算法,如自聚焦算法等^[21-23]。

PSOCT系统中的重要功能是可以探测到两正交方向上的干涉光谱^[7]。通常情况下,将干涉信号通过偏振分束器分离,使用两个光谱仪即可实现正交方向上信号的同时探测。但对于双光谱仪的PSOCT系统,体积大不利于小型化,且需要对两通道进行高精度的配准和同步触发,搭建费用较高,硬件控制难度大。由于相机的不同造成的灵敏度差异,致使后期光谱矫正难度大,因此需要研究基于单相机的PSOCT系统。基于单相机的偏振OCT探测拥有多种不同的实现方式,一般分为分时探测和实时探测,分时探测的主要原理是使用单相机在不同时间分别探测两正交方向上的干涉信号,而实时探测一般是实现相机的分区域探测,在同一时刻将不同方向上的干涉信号接收在相机的不同区域。

对于分时探测,2009年LEE S W等使用光开关对正交偏振光谱进行选择^[24],使其在相邻时间入射至同一光谱仪中,减少了因光谱仪配准精度不高带来的问题。除了对干涉信号进行正交方向的分离方法外,HEY W等使用光快门和光开关两种方式对参考臂返回光的正交偏振态进行连续切换选择,同样实现了样本的偏振信息测量^[25-26]。对于实时探测,可以将两正交方向干涉信号以不同角度入射同一光栅,以不同出射角被相机相邻区域探测接收^[27]。另外,根据沃拉斯顿棱镜对正交偏振态分离的作用,将干涉信号通过沃拉斯顿棱镜后即可在相机不同区域得到不同偏振态的干涉信号^[28]。由于光栅衍射效率随角度的变化以及相机阵列灵敏度的差异,使得这两种方法需要对信号进行矫正匹配。同时因为成像像素值的减半,限制了系统成像范围,使用多阵列相机可以解决该问题^[29]。

本文提出了一种单相机微米谱域偏振OCT系统,使用偏振片在不同时间选通两正交偏振干涉光谱。共用同一光谱仪,减少由于相机不匹配带来的光谱畸变问题。系统使用宽带光源实现高分辨率性能,从硬件软件两方面补偿系统色散。利用单输入态偏振光计算重构得到生物组织强度图和相位延迟图像,展现系统成像质量,证明了偏振测量的可行性。

1 系统结构和原理

1.1 PS-SDOCT系统

基于单相机的微米分辨PS-SDOCT系统原理结构如图1,部分装置实物如图2。超连续激光光源(SC480-2, Fianium, UK)的出射光谱波长范围在410~2400 nm,经过二向色镜(DMLP100T, Thorlabs, US)后,波长1 μm以内的光反射通过偏振片P₁,使经过L₂进入单模光纤耦合器的光为垂直线偏振光。其余光被光挡吸收以防止对人眼的伤害和对环境的影响。进入参考臂的光通过L₃准直入射至平面反射镜,参考

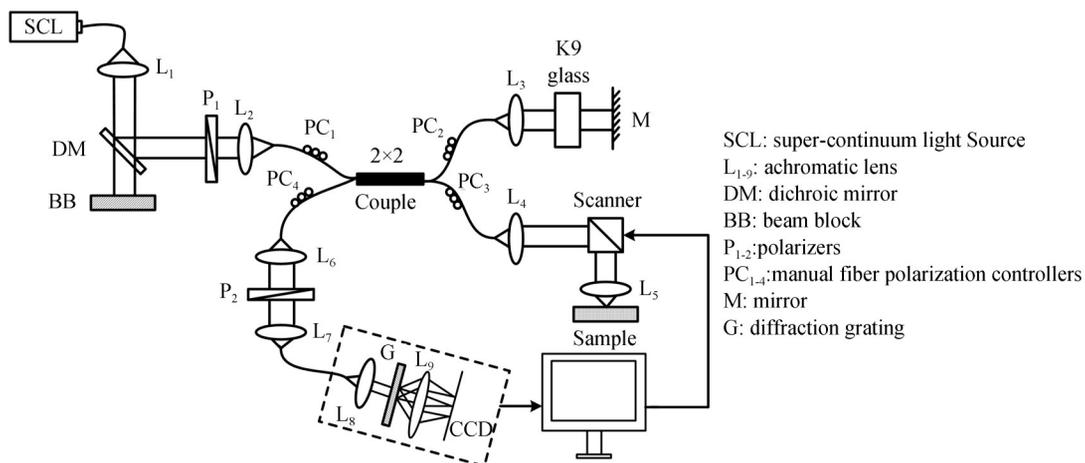


图1 单相机微米 PS-SDOCT 系统结构

Fig.1 System structure of micron resolution PS-SDOCT using a single camera

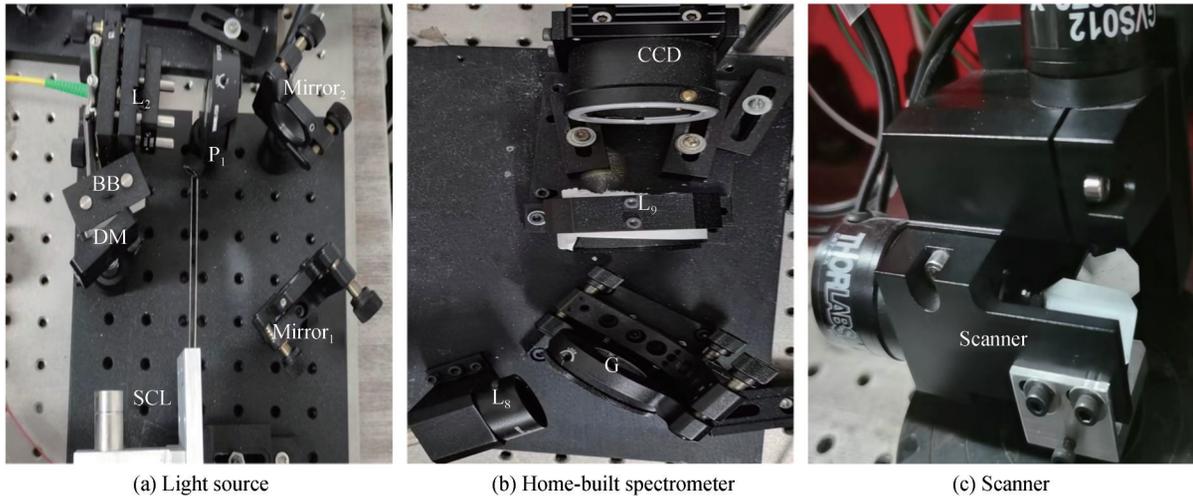


图2 实验系统部分装置实物
Fig.2 Parts of the experimental system

臂中加入了K9光学玻璃以补偿系统色散。进入样品臂的光通过 L_4 准直入射至X-Y扫描振镜(GVS012, Thorlabs, US),然后通过 L_5 的物镜入射至样品表面。两臂返回的信号于单模光纤耦合器发生干涉,干涉信号通过 L_6 入射至空间光路,通过偏振片 P_2 进行正交偏振态光谱信号的选择,某一时刻只有水平或垂直偏振光谱通过 L_7 入射至自制光谱仪中,如图1中虚线框内结构。偏振分离后的干涉信号通过 L_8 入射至透射式光栅(1002-1, Wasatch, US)进行分光,再由 L_9 汇聚至CCD(EM4, e2v, UK)接收。经过标定,系统最终成像波长范围在760~920 nm,中心波长为840 nm,空气中理论分辨率达到1.95 μm ,经色散补偿后,在折射率为1.4的生物组织中实际轴向分辨率为1.61 μm 。

系统偏振态由起偏器 P_1 和四个手动偏振控制器(PC)进行调制,偏振控制器能够作用在光纤的全工作波段,基于应力双折射改变单模光纤中光的偏振态。在基于单模光纤的PSOCT系统中,样品的偏振特性会受到光纤双折射的影响,由于光纤组件的偏振特性未知,为了准确提取样品的偏振特性,需要通过调节偏振控制器使得偏振控制器和单模光纤的综合作用等效于空间光路中的四分之一波片或全波片^[30]。在实际操作中,首先调整 PC_1 ,使得入射至参考臂和样品臂的光功率最大。其次调整 PC_3 ,需要控制入射至样品表面的光为圆偏振光,使用四分之一波片和偏振片的组合对样品臂出射的光进行偏振态检测。之后将参考臂遮挡,使用平面镜作为样品,调整 PC_4 ,转动 P_2 切换通道,观察两通道的光谱功率,使得一通道下光功率最大,另一通道下光功率最小。最后遮挡样品臂,调整 PC_2 ,转动 P_2 切换通道,观察两通道下的光谱功率,使得其大小保持相同^[31]。

1.2 系统色散补偿

对于光纤型SDOCT系统,由于光纤带有较强的色散,所以在光纤干涉式系统中,两臂光纤长度差和光学元件通常会引入色散不匹配问题。这里通过硬件和软件两方面对系统色散进行补偿。对于硬件补偿,除了光学透镜都使用消色差透镜外,在参考臂中加入K9光学玻璃片。由于很难计算所需精确的玻璃厚度值,使用不同厚度的玻璃块依次加入,以平面镜为样品,通过强度图和点扩散函数曲线,观察不同厚度的补偿结果,选取最合适的厚度。

在后续处理中,采用数值补偿的方法对系统色散进行补偿。由于重建样品结构需要对波数 k 进行傅里叶变化,而相机获取到的干涉信号是有关波长的函数,需要进行重采样使其映射到 K 空间。色散不匹配引入的是相位的影响,其中相位与多阶色散的关系可以由泰勒级数展开表达^[32],即

$$\theta(k) = \theta(k_0) + \left. \frac{\partial\theta(k)}{\partial k} \right|_{k_0} (k_0 - k) + \frac{1}{2} \cdot \left. \frac{\partial^2\theta(k)}{\partial k^2} \right|_{k_0} (k_0 - k)^2 + \dots + \frac{1}{n} \cdot \left. \frac{\partial^n\theta(k)}{\partial k^n} \right|_{k_0} (k_0 - k)^n \quad (1)$$

式中, k_0 为中心波数,泰勒展开式中,第一项为固定相位因子,第二项系数表示群延迟,这两项与色散展宽没有关系。第三项系数表示一阶色散,第 n 项系数表示 $n-1$ 阶色散。为了补偿色散,对获取到的干涉信号减

去Bscan中所有Ascan的均值以去除直流项,希尔伯特变换后根据式(1)进行相位拟合得到当前各阶色散系数,随后进行数值模拟重新确定系统消色散后的各阶系数,使用傅里叶变换重构补偿色散后的样品结构信息。

1.3 偏振模型和计算

对于偏振片P,两正交通道下的琼斯矩阵为

$$J_{PH} = \begin{bmatrix} 1 & 0 \\ 0 & 0 \end{bmatrix} \quad (2)$$

$$J_{PV} = \begin{bmatrix} 0 & 0 \\ 0 & 1 \end{bmatrix} \quad (3)$$

式中,下标PH和PV分别表示水平和垂直偏振光分量的通道,从偏振片的矩阵中可知,光经过偏振片后,两垂直通道的信息将被分离,且并不会引入额外的相位延迟信息。探测到的两时间段内相互垂直的偏振光谱信号可表示为

$$\widetilde{A}_H(z) = A_H(z) e^{i\phi_H(z)} \quad (4)$$

$$\widetilde{A}_V(z) = A_V(z) e^{i\phi_V(z)} \quad (5)$$

式中, $\phi(z)$ 和 $A(z)$ 分别表示两方向沿深度上的相位和幅值。使用斯托克斯矢量来表示返回光的偏振状态^[33],可表示为

$$S = \begin{bmatrix} S_0 \\ S_1 \\ S_2 \\ S_3 \end{bmatrix} = \begin{bmatrix} \widetilde{A}_H \widetilde{A}_H^* + \widetilde{A}_V \widetilde{A}_V^* \\ \widetilde{A}_H \widetilde{A}_H^* - \widetilde{A}_V \widetilde{A}_V^* \\ \widetilde{A}_H \widetilde{A}_V^* + \widetilde{A}_V \widetilde{A}_H^* \\ i(\widetilde{A}_H \widetilde{A}_V^* - \widetilde{A}_V \widetilde{A}_H^*) \end{bmatrix} = \begin{bmatrix} A_H^2 + A_V^2 \\ A_H^2 - A_V^2 \\ 2A_H A_V \cos \Delta\phi \\ 2A_H A_V \sin \Delta\phi \end{bmatrix} \quad (6)$$

式中, $\Delta\phi = \phi_H - \phi_V$ 。

对于生物组织的琼斯矩阵 J_{sam} ,在不考虑二向色性的前提下,可以将其分解为两个旋转矩阵和一个正交相位延迟矩阵的乘积形式^[34]

$$J_{sam} = R(\theta) \mathbf{A}(\delta) R(-\theta) = \begin{bmatrix} \cos \theta & -\sin \theta \\ \sin \theta & \cos \theta \end{bmatrix} \begin{bmatrix} e^{i\delta/2} & 0 \\ 0 & e^{-i\delta/2} \end{bmatrix} \begin{bmatrix} \cos \theta & \sin \theta \\ -\sin \theta & \cos \theta \end{bmatrix} \quad (7)$$

式中, θ 表示光轴方向, δ 表示相位延迟,对式(7)进一步计算可得

$$J_{sam} = \begin{bmatrix} e^{i\delta/2} \cos^2 \theta + e^{-i\delta/2} \sin^2 \theta & (e^{i\delta/2} - e^{-i\delta/2}) \cos \theta \sin \theta \\ (e^{i\delta/2} - e^{-i\delta/2}) \cos \theta \sin \theta & e^{i\delta/2} \sin^2 \theta + e^{-i\delta/2} \cos^2 \theta \end{bmatrix} \quad (8)$$

当系统参考臂返回45°线偏振光,入射样品表面光为圆偏振态光时,根据出射琼斯矢量计算后转化为斯托克斯矢量后,式(6)可表示为^[14]

$$S = \begin{bmatrix} S_0 \\ S_1 \\ S_2 \\ S_3 \end{bmatrix} = R(z) \begin{bmatrix} 1 \\ -\sin \delta(z) \sin 2\theta(z) \\ \sin \delta(z) \cos 2\theta(z) \\ \cos \delta(z) \end{bmatrix} \quad (9)$$

样品的强度信息和相位延迟可通过式(6)、(7)计算,分别表示为

$$I(z) = |A_H(z)|^2 + |A_V(z)|^2 \quad (10)$$

$$\delta(z) = \arccos^{-1} \frac{S_3}{S_0} \quad (11)$$

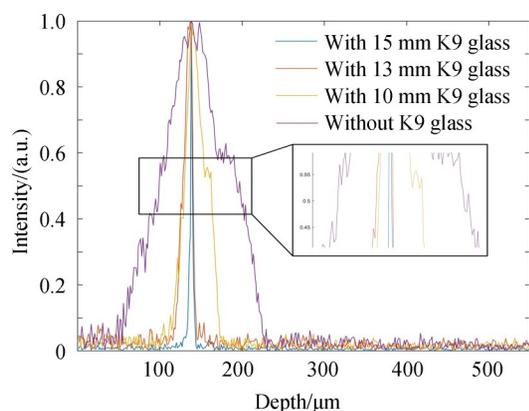
2 实验结果

2.1 色散补偿

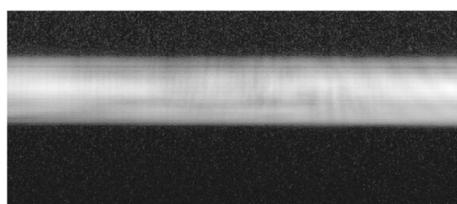
使用PS-SDOCT系统,旋转 P_2 使其固定在0°方位,对此时获取到的光谱数据进行处理分析。对于硬件补偿,以平面镜作为样品,依次插入三种不同厚度的K9玻璃,为了更直观地看出玻璃厚度对色散的影响,计

算不同厚度玻璃补偿下的点扩散函数,观察其半高宽,如图3(a)中插图。

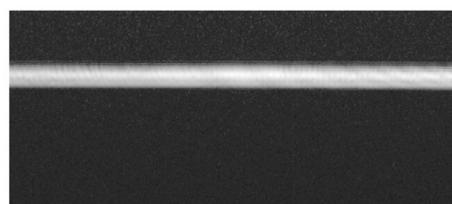
从图3(b)可看出,当不加入玻璃时,平面镜成像结果最差,随着玻璃厚度的增加,色散补偿效果越明显,当玻璃厚度在15 mm时,平面镜成像效果较为理想。



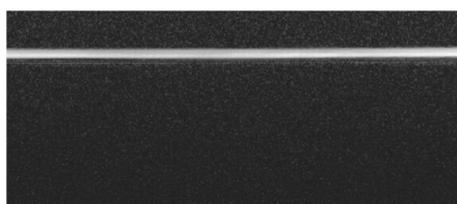
(a) Point spread function under different thicknesses of K9 glass



(b) Intensity image of mirror without K9 glass compensation



(c) Intensity image of mirror with 10 mm K9 glass compensation



(d) Intensity image of mirror with 13 mm K9 glass compensation



(e) Intensity image of mirror with 15 mm K9 glass compensation

图3 基于硬件色散补偿结果

Fig.3 Results based on hardware dispersion compensation

继续叠加参考臂中玻璃厚度,当插入K9玻璃厚度为17 mm时,成像结果如图4,此时点扩散函数半高宽展宽,因此基于硬件补偿的结果选定15 mm的K9玻璃厚度。

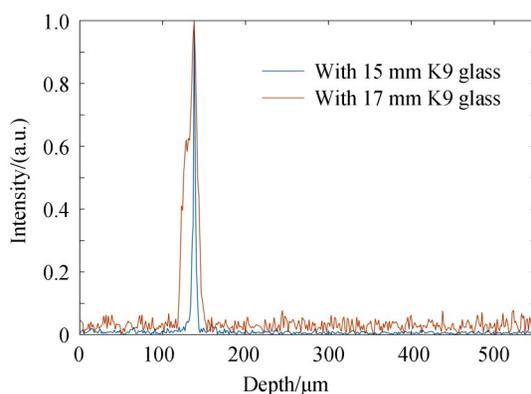


图4 15 mm和17 mm K9玻璃下的平面镜成像点扩散函数
Fig.4 Point spread function under 15 mm and 17 mm K9 glass

为了更好地展示基于数值补偿的色散结果,在参考臂中插入 13 mm 的 K9 玻璃,以平面镜为样品,首先拟合色散相位曲线,结果如图 5(a)。求解出补偿后的各阶色散系数,观察平面镜补偿前后的效果。这里对一阶色散和二阶色散进行补偿。补偿后,计算各阶色散补偿下的点扩散函数,结果如图 5(b),从点扩散函数的半高宽中可以看出,相较于红色曲线未进行补偿的结果,一阶补偿(绿色)和二阶补偿(蓝色)效果呈现递增趋势。

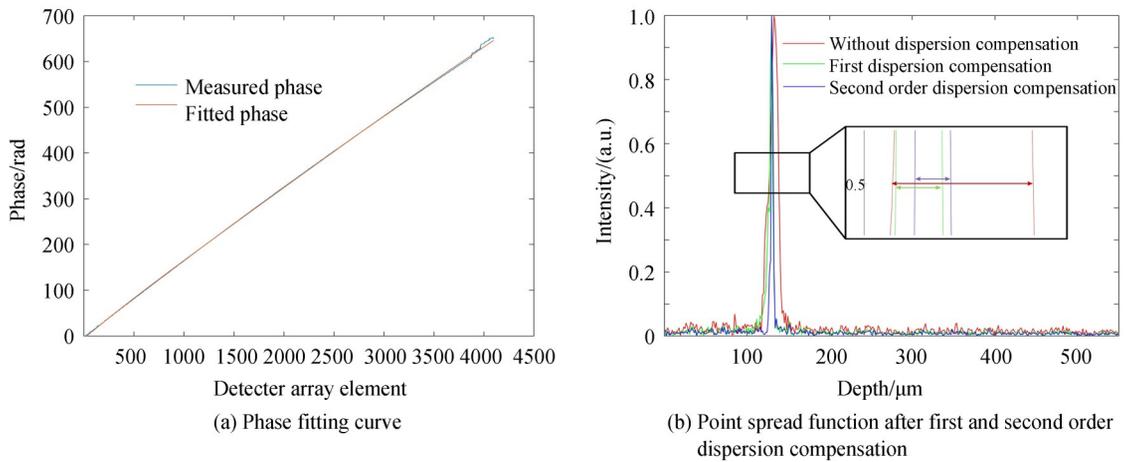


图 5 基于数值补偿的色散结果
Fig.5 Dispersion results based on numerical compensation

为了验证系统的成像性能,自制钛粉胶质溶液,其中钛粉为二氧化钛颗粒,胶选用聚二甲基硅氧烷 (Polydimethylsiloxane, PDMS),使用 2 g 钛粉和 4mL PDMS 充分搅匀,凝固后呈透明状硅胶质地。对数值补偿前后的结果图进行对比分析。从图 6(a)中可以明显看出,未补偿前的结构表面展宽较为严重,钛粉结构有沿深度方向的拉伸。对结果进行二阶色散补偿结果如图 6(b),胶质溶液表面信息变薄,分辨率提高。从色散补偿前后相同位置处的结构放大图中可以观察到,钛粉颗粒结构在补偿后有了明显的变化,大小保持相对均匀。胶质内其余钛粉颗粒结构亦有所提升。

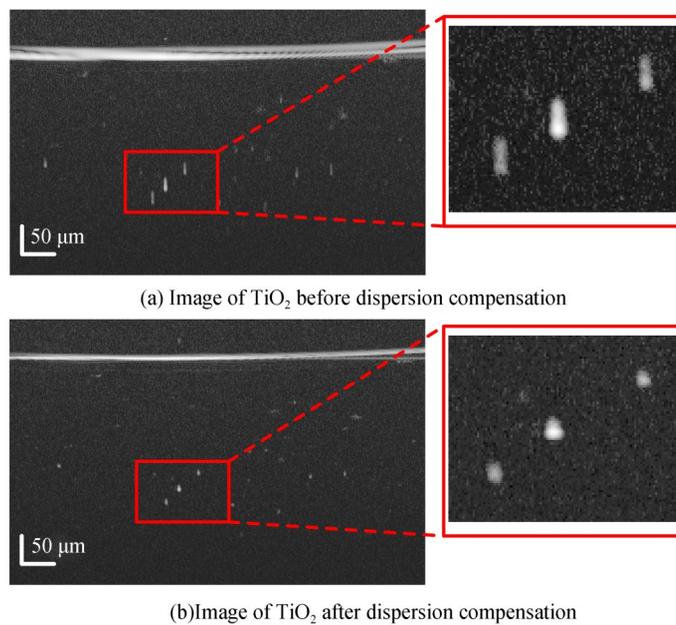


图 6 色散补偿前后的钛粉颗粒结构
Fig.6 TiO₂ before and after dispersion compensation

2.2 偏振成像结果

为了验证系统的偏振特性成像性能,首先使用标准四分之一波片作为样品测量其相位延迟数据,平均相位延迟值约 85° 。

为了进一步证明该系统的性能并说明其应用前景,对离体生物组织进行偏振成像,使用新鲜的牛腱肉作为样品,沿牛腱表面方向制作薄层切片。两通道分别采集 10 帧 B 扫图,每帧 B 扫含 1 024 帧 A 扫,数据采集在 10 s 内完成。实验结果如图 7。

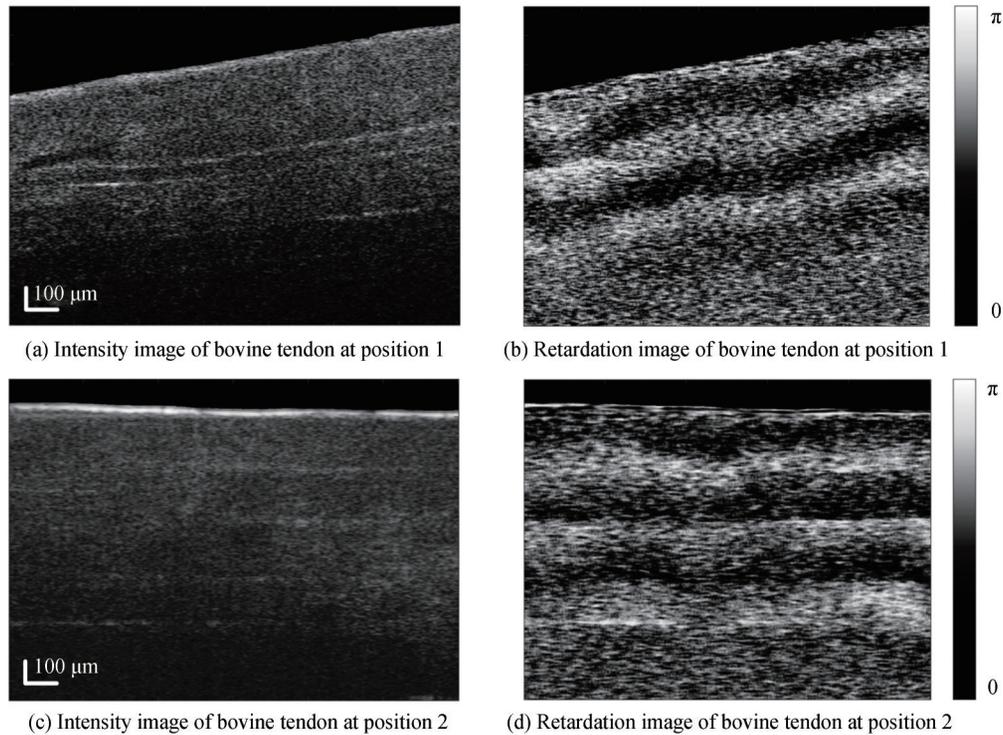


图 7 离体牛腱组织的 PS-SDOCT 图

Fig.7 The PS-SDOCT images of bovine tendon in vitro

从图 7(a)和(c)能够明显看出牛腱肉内部的层状纤维结构。图 7(c)中表面的亮线是由于新鲜牛腱表面的强散射造成的。通过公式(11)对相应部位进行相位延迟的计算成像,结果如图 7(b)、(d),分别对应 7(a)和(c)中的位置。该结果可以明显反应出光在组织内沿深度方向上的相位延迟成周期性变化,且条纹走向与组织表面平行,这是强度图中无法分辨出的。部分条纹的弯曲可能是由于激光长时间照射导致组织温度变化或者组织内部原有的损伤而形成。其计算结果与文献报道结果一致^[35],验证了该基于单相机的偏振系统测量的准确性。

3 结论

本文提出了一种基于单相机的微米分辨谱域偏振光学相干层析成像系统,系统基于光纤光路,使用偏振片对正交光谱进行选择,无需进行复杂的机械结构设置和相机配准。能够通过简单的操作实现 PSOCT 系统和 SDOCT 系统间的转换。通过硬件和软件两方面对系统由于光源带宽较大和光学器件的不匹配引入的色散进行补偿矫正,补偿至二阶色散。实验结果表明矫正后的系统分辨率和成像质量有了明显的提高和改善,并且当系统色散较为严重时,先考虑硬件补偿方法,当硬件补偿得到明显的改善后,再使用软件上的算法补偿。建立偏振计算模型,使用 Stokes 矢量计算得到组织的强度图和相位延迟图,对新鲜牛腱肉的不同位置进行偏振成像,观察到牛腱内部的纤维层状结构和沿深度方向成周期性变换的相位延迟图像,结果有效证明了基于单相机的 PSOCT 系统的可行性。研究为实验室中分析组织偏振信息提供了新的方法。

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Micron Polarization-sensitive Spectral-domain Optical Coherence Tomography Based on Single Camera

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Abstract: Optical Coherence Tomography (OCT) is an interferometric imaging method, and it is mostly used in the field of imaging for its non-invasive, high-resolution and high-speed properties. This technology can also be applied to detect defects in materials. Although OCT can provide images of morphological structure, it can not distinguish tissues with similar light intensity properties in pathologies. Polarization-sensitive Optical Coherence Tomography (PS-OCT) is an imaging system extended from conventional OCT, enabling functional imaging. It can use Stokes parameters, Jones and Muller matrices to calculate the polarization properties of the samples, like the birefringence phase retardation, the optic axis orientation and depolarization. PS-OCT has been used in a number of medical applications, such as burn depth determination and tumor yield assessment. And it also can be applied to examination of stress-induced birefringence of materials. In previous systems, it is required to detect the two orthogonally polarized components by using dual cameras based spectrometers. But there are problems with this system arrangement. For example, it has high cost and requires complex hardware and software designs. In addition, it is hard to achieve the uniform detection for two cameras, the mismatch between the two channels can lead to polarization distortions and failure to calculate the true information and additional algorithms are necessary to tackle it. So a series of single cameras based methods have been proposed. Single cameras based systems can achieve time-sharing detection or real-time detection of two orthogonal channels with relatively lower cost and simpler system setup. Wollaston prism, optical switch, grating and multi-camera are often used to achieve single camera detection. Improving the axial resolution of the system can enable it to have more potential applications. The OCT system with micron axial resolution can

achieve cellular and subcellular level imaging and detect subsurface defects in ceramics or other materials. In order to achieve such a high axial resolution, super-continuum light source is generally used to increase the bandwidth of imaging. Because the optical path of the reference arm and the sample arm are not completely symmetrical, as the spectral bandwidth increases, the system can introduce serious dispersion and affect axial resolution. In this paper, we demonstrate a polarization-sensitive spectral domain optical coherence tomography imaging system using a single camera with micron axial resolution. It is an all single-mode fiber-based system, and a broad bandwidth light source is used to achieve micron axial resolution. In order to increase actual axial resolution of the system, the system dispersion effects are compensated by using both hardware and software methods. After compensating the first order and second order dispersion, the measured axial resolution of the system is about $1.61\ \mu\text{m}$ for the sample with an approximated refractive index of 1.4. In order to realize the measurement of the polarization state of the light reflected from the sample, the polarization state of light incident on the sample surface and the reference arm is modulated by the polarizer and four polarization controllers. The horizontal and vertical polarization interference signals are separately measured via channel switching of a polarizer and they can achieve time-sharing detection by using only one camera. Intensity and phase retardation information of the samples can be calculated by the signals obtained from the two channels at different times. To verify the capability of our system to measure the polarization information, we succeed in polarization imaging with a single camera and obtaining the images of intensity and polarization parameter contrast of the biological tissue in vitro by using Stokes vector. From the retardation image of bovine tendon at different position, it can be obviously observed that the phase retardation varies periodically with the increase of the depth in the tissue. This method is characterized by its simple system arrangement and lay the basis for miniaturizing in vivo high resolution polarization parameter imaging.

Key words: Biomedical imaging; Polarization-sensitive optical coherence tomography; Single camera; Dispersion compensation

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