

## 基于共定位相位成像的白细胞亚结构特征参数研究

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**摘要** 白细胞分类在血液分析、临床疾病的诊断和治疗中具有重要意义。人工镜检作为血细胞分析的金标准,耗时较长且高度依赖检测人员的经验。定量相位成像可测量细胞各处的相位分布,是研究细胞形态学和生物化学特征的有效方法。利用基于数字全息显微和明场显微成像的共定位相位成像系统对健康人外周血涂片中的 5 种白细胞进行研究,定量分析了不同白细胞及其亚结构中的相位分布情况,提取出多个有效辅助白细胞分类的特征参数,并进一步分析了镜检中容易混淆的异型淋巴细胞。结果表明,利用提取的细胞亚结构特征参数可对白细胞进行有效分类,也能较好区分异型淋巴细胞。因此,基于共定位相位成像的细胞亚结构特征参数可为白细胞分类、各类血液疾病的诊断和治疗提供依据和参考,且此种方法无须再对常用的染色样品进行特殊处理,应用场景众多。

**关键词** 生物光学; 定量相位成像; 数字全息; 白细胞亚结构; 相位分布特征

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## 1 引言

白细胞是人体免疫系统的主要载体,种类多样、形态多变,其分类计数和形态结构特征在血液分析检测和临床疾病诊疗中十分重要,是细菌感染<sup>[1]</sup>、病毒感染<sup>[2]</sup>、过敏性疾病<sup>[3]</sup>、癌症<sup>[4-5]</sup>等一系列疾病的主要甚至唯一确诊手段。明场显微镜下人工镜检<sup>[6]</sup>是血细胞分析的传统检验方法,准确率高且能对细胞形态作出准确判断,但是该方法高度依赖检测人员经验,速度慢、劳动强度大,不利于基层医院推广应用。随着技术的发展,近年来各种全自动血细胞分析仪被广泛用于白细胞检测,如基于荧光标记的流式细胞术<sup>[7]</sup>。但由于白细胞结构的多变性,其结果存在一定的假阳性,需要人工镜检复查<sup>[8]</sup>,且仪器昂贵。

数字全息显微术(DHM)是一种无标记的定量相位成像技术,已广泛用于各种生物细胞的研究<sup>[9-10]</sup>。Shu等<sup>[11]</sup>利用DHM研究了未染色的白细胞,结合深度学习,对粒细胞、单核细胞、淋巴细胞的分类准确率达到80%以上,但由于三种粒细胞的相位特征不明显,无法进行进一步的区分。光学衍射层析成像技术(ODT)可利用多角度信息实现生物细胞的三维折射率分布重构<sup>[12-14]</sup>。2021年,Ryu等<sup>[15]</sup>利用ODT研究了三种亚型淋巴细胞的三维折射率特征,结合深度学习,对单核细胞、B淋巴细胞、T淋巴细胞的识别率达到96%。2022年,Kim等<sup>[16]</sup>利用ODT研究了哮喘患者

的嗜酸性粒细胞的折射率变化,为哮喘的诊断和治疗提供了一个新的思路。ODT光路较复杂,且多角度信息的获取限制了成像视野(FOV),不利于大范围的血液检测。除此之外,Ojaghi等<sup>[17]</sup>基于核酸和蛋白质不同波长的吸收峰,利用深紫外光源对血细胞进行成像,并采用算法将灰度多光谱紫外图像转换为彩色图像,准确模拟出瑞氏染色的结果。Chen等<sup>[18]</sup>基于相衬技术,采用415 nm光源增强细胞器的对比度,实现了血细胞的无标记分类,该方法光路结构简单,分类准确率较高。然而常用显微镜的器件是针对可见光波段的,不易与紫外光源集成。

上述方法无需染色步骤,针对白细胞固有的相位特征进行研究,在实验室少量样本的条件下可实现较高准确率的白细胞分类,但还没有实现实际应用。

人工镜检是白细胞分类的金标准,其准确率依赖检测人员经验。针对易混淆的细胞,检测人员通常以健康人外周血中白细胞占比作为判断依据,极易造成误判,此时其他辅助判断方法显得尤为重要。目前深度学习已经被广泛用于白细胞分类<sup>[19-20]</sup>,能够在一定程度上降低对检测人员的技术要求,但其本质还是利用染色细胞的形貌和颜色特征来作判断。为了提高白细胞分类的准确率,除了要保证涂片、染色等过程的均一性外,通常还须在高倍显微镜下进行图像采集和处理,耗时长、数据量大、训练时间长。卷积神经网络

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在 100 倍镜下对白细胞分类的准确率可达 95% 左右, 当采用 40 倍镜时, 分类准确率会下降<sup>[21]</sup>。人工镜检和深度学习方法均是基于染色血片的显微图像进行判断, 并不关注细胞的相位信息。2019 年, Majeed 等<sup>[22]</sup>对染色的乳腺癌组织进行定量相位成像研究, 发现可以从相位中得到明场图像无法获得的信息, 即胶原纤维方向信息, 从而有效预测后续乳腺癌的发展情况。基于染色细胞组织的定量相位成像研究结果表明, 相位信息可提供与明场图像不同的有效信息。而基于无标记的白细胞的固有相位特征进行分类, 非特异性成像的本质使其对相位特征不明显的白细胞的分辨准确率较低<sup>[11]</sup>。

针对非特异性成像方法在白细胞分类中的问题, 本文构建了基于 DHM 和明场显微成像的共定位相位成像系统, 利用明场下染色白细胞中细胞核与细胞质的高对比度, 研究分析了不同白细胞亚结构中的相位特征, 总结了 5 种亚型白细胞的相位分布特点, 同时提取出有效辅助白细胞分类的特征参数, 在此基础上进一步研究了镜检中容易混淆的异型淋巴细胞。研究结果表明, 本方法可对整体相位特征不明显的三种粒细胞进行分类, 分类准确率高达 94%, 对异型淋巴细胞的识别准确率达 84.5%。本方法可直接使用临床镜检中使用的常规染色血涂片样品, 同时易与显微系统集成, 有广阔的应用前景。

## 2 研究方法

### 2.1 系统光路

为了同时获得白细胞的明场图像和定量相位信息, 我们搭建了基于 DHM 和明场显微成像的共定位成像系统。其原理如图 1 所示。其中 DHM 采用的系统光源为波长为 650 nm 的半导体激光, 经过空间滤波后, 其被分束棱镜 1 (BS1) 分为物光和参考光。物光经反射镜 M1 反射后照射样品, 再经过显微物镜 (40 $\times$ , 数值孔径 NA=0.65) 和成像透镜, 在互补金属氧化物半导体 (CMOS) 相机上与参考光发生干涉, 得到样品的全息图。明场显微成像系统的光源为宽谱发光二极管 (LED), 光束整形后通过反射镜对样品照明, 再经同一套显微物镜和成像透镜, 在相同的相机上成像。反射镜 M3 被安装在一个可翻转支架上, 通过翻转支架可以将 M3 置于光束路径上或者移出光束之外, 无须改变其余光路即可实现光路在两个成像系统中的灵活切换。这样就获得了白细胞的全息图和原位明场像。利用单次测量得到的离轴全息图, 通过重构算法<sup>[23]</sup>进行傅里叶重构, 即可重构出白细胞的定量相位分布信息。虽然在 40 倍镜的精度下无法得到 100 倍镜明场成像的高分辨率, 无法看清细胞内的微小颗粒, 但细胞质中这些小颗粒带来的整体相位变化对于相位信息及相位特征的提取是至关重要的。

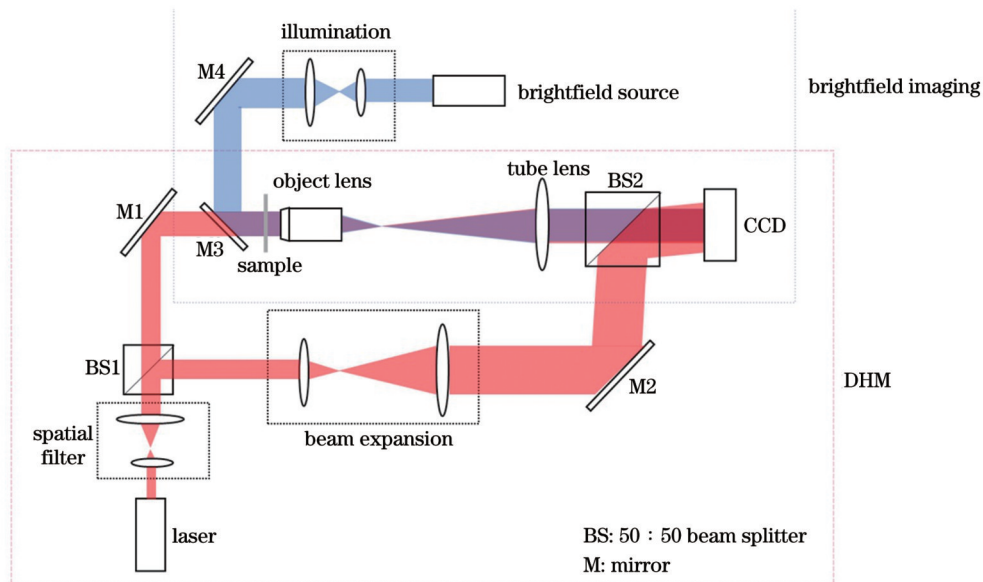


图 1 共定位成像系统示意图

Fig. 1 Schematic of co-localized imaging system

### 2.2 基于原位明场图像的细胞分割

在基于相位特征信息提取的白细胞分类中, 细胞质(或细胞核)中的大量细胞器对细胞质(或细胞核)整体的相位影响十分重要, 因此可将每一个白细胞分割成细胞核与细胞质两部分, 以实现相位特征参数的分析和提取。然而 DHM 非特异性成像的特点使得直接

分割相位信息十分困难, 因此本文采取先分割明场图像, 再将细胞亚结构的位置信息转印到原位定量相位图中的方法, 实现对白细胞相位信息的分割。首先将明场图像中的单个白细胞提取出来, 再将其分割成细胞核与细胞质两部分, 最后将明场图像中获得的白细胞及其胞核、胞质的位置信息迁移到相位图中, 获得单

个白细胞的定量相位分布以及对应细胞核、细胞质的相位分布。

### 2.3 特征参数提取

基于单个白细胞的定量相位分布以及对应细胞核、细胞质的相位分布情况,可分析提取出一系列特征参数用于定量描述和分析不同白细胞的形态学及亚结构中的相位变化特征。具体特征参数名称及定

义如表 1 所示。除了较常用的描述细胞形态学特征参数外,这里特别提出了若干描述细胞核、细胞质的相位特征及其相对关系的参数。例如核质相位不匹配度( $D$ )表征细胞核相位与细胞质相位之间的大小关系;细胞核梯度分布( $\nabla_N$ )与细胞质梯度分布( $\nabla_C$ )表征相位变化的剧烈程度,可反映细胞质中颗粒的分布情况。

表 1 特征参数  
Table 1 Feature parameters

Parameter	Definition
Projection area ( $S$ )	Projected area along direction of light
Cell circularity ( $\Phi$ )	Variance of distance from cell contour to cell center, $\Phi = \frac{\sum_{i=1}^{N_{\text{edge}}} \left\{ \sqrt{[x_{\text{edge}}(i) - x_{\text{central}}]^2 + [y_{\text{edge}}(i) - y_{\text{central}}]^2} \right\}}{N_{\text{edge}}}$
Circumference-area ratio ( $R$ )	Ratio of nucleus circumference to area, $R = \frac{S_{\text{nucleus}}}{C_{\text{nucleus}}}$
Peak-valley phase difference ( $\Delta\varphi_{\text{max}}$ )	Difference between maximum and minimum phases of cell, $\Delta\varphi_{\text{max}} = \varphi_{\text{max}} - \varphi_{\text{min}}$
Nucleus-cytoplasm phase difference ( $\Delta\varphi_{\text{mean}}$ )	Average phase difference between nucleus and cytoplasm, $\Delta\varphi_{\text{mean}} = \bar{\varphi}_{\text{nucleus}} - \bar{\varphi}_{\text{cytoplasm}}$
Nucleus-cytoplasm phase mismatch degree ( $D$ )	Number of pixels in cytoplasm whose phase is greater than average phase of nucleus
Cell phase variance ( $\sigma_{\text{cell}}$ )	Variance of phase in whole cell, $\sigma_{\text{cell}} = \frac{\sum_{i=1}^{N_{\text{cell}}} \sqrt{[\varphi_{\text{cell}}(i) - \bar{\varphi}_{\text{cell}}]^2}}{N_{\text{cell}}}$
Cytoplasm phase variance ( $\sigma_c$ )	Variance of phase in cytoplasm, $\sigma_c = \frac{\sum_{i=1}^{N_{\text{cytoplasm}}} \sqrt{[\varphi_{\text{cytoplasm}}(i) - \bar{\varphi}_{\text{cytoplasm}}]^2}}{N_{\text{cytoplasm}}}$
Nucleus gradient distribution ( $\nabla_N$ )	Ratio of nucleus whose fluctuation is more intense (gradient is greater than 1)
Cytoplasm gradient distribution ( $\nabla_C$ )	Ratio of cytoplasm whose fluctuation is more intense (gradient is greater than 1)

在表 1 中: $\varphi_{\text{max}}$  为细胞相位最高值; $\varphi_{\text{min}}$  为细胞相位最低值; $\varphi_{\text{cell}}$  为细胞相位; $\bar{\varphi}_{\text{cell}}$  为细胞平均相位; $\varphi_{\text{cytoplasm}}$  为细胞质相位; $x_{\text{edge}}$  和  $y_{\text{edge}}$  分别为细胞轮廓上点的横、纵坐标; $x_{\text{central}}$  和  $y_{\text{central}}$  分别为细胞几何中心点的横、纵坐标; $N_{\text{cell}}$  和  $N_{\text{cytoplasm}}$  分别为细胞和细胞质的像素点个数; $N_{\text{edge}}$  为细胞轮廓上的像素点个数; $i$  为像素点编号; $C_{\text{nucleus}}$  和  $S_{\text{nucleus}}$  分别为细胞核的面积和周长所占像素点的个数; $\bar{\varphi}_{\text{nucleus}}$  和  $\bar{\varphi}_{\text{cytoplasm}}$  分别为细胞核与细胞质的平均相位。

## 3 分析与讨论

### 3.1 共定位成像结果对比分析

血液中的白细胞分为中性粒细胞(neutrophil)、嗜酸性粒细胞(eosinophil)、嗜碱性粒细胞(basophil)、单核细胞(monocyte)和淋巴细胞(lymphocyte)这 5 类。利用共定位成像系统对瑞氏染色后的健康人外周血涂

片进行成像,图 2 为实验测得的 5 种不同白细胞的明场图像及原位重构的相位分布情况。明场图像中细胞核与细胞质的高对比度可提供有效的细胞位置结构信息,用于细胞共定位相位信息的提取和分割。

完成细胞相位信息的提取和分割后,对比分析多个不同白细胞,发现其特征各不相同:淋巴细胞面积最小;单核细胞面积较大且外形较不规则;三种粒细胞的形态特征差异较小。在相位分布特征方面:中性粒细胞的细胞核相位明显大于细胞质;嗜酸性粒细胞中细胞核相位与细胞质相位类似;嗜碱性粒细胞中细胞质的相位波动较大。

### 3.2 特征参数统计对比分析

为了更全面地对比研究 5 种不同白细胞及其亚结构的特征,对大量不同种类的白细胞样本进行统计分析。由于血液中不同白细胞的占比差异很大,为保证统计样本容量,在染色血片中选择中性粒细胞、嗜酸性粒细胞、嗜碱性粒细胞、单核细胞、大淋巴细胞和小淋

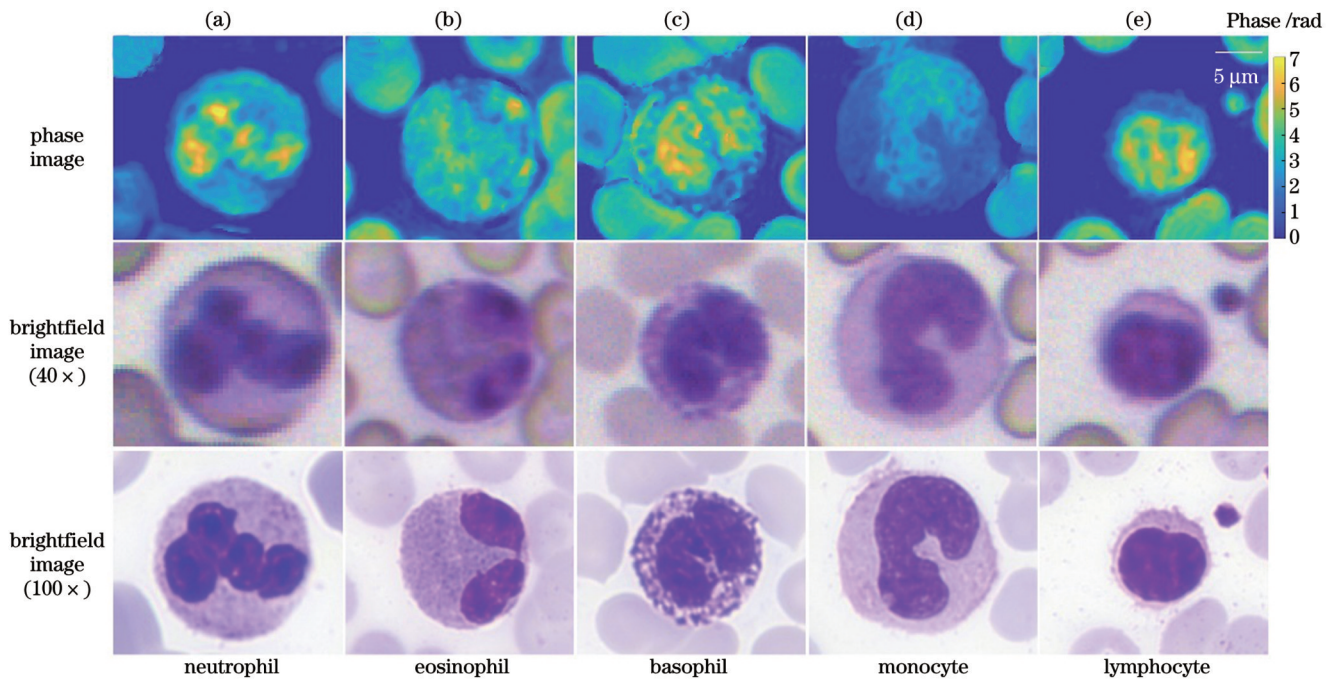


图 2 5 种白细胞的相位图及其对应的明场图。(a)中性粒细胞;(b)嗜酸性粒细胞;(c)嗜碱性粒细胞;(d)单核细胞;(e)淋巴细胞  
Fig. 2 Phase images of five types of white blood cells and corresponding brightfield images. (a) Neutrophil; (b) eosinophil; (c) basophil; (d) monocyte; (e) lymphocyte

淋巴细胞各 100 个进行共定位成像。这里根据细胞的大小和细胞质含量的多少,将淋巴细胞分为小淋巴细胞 [lymphocyte (S)] 和大淋巴细胞 [lymphocyte (L)]。在此基础上,基于明场图像对原位相位信息进行提取和分割,形成包含 600 个不同白细胞及其亚结构的相位样本数据集。

首先对比研究投影面积  $S$ 、细胞圆度  $\Phi$  以及细胞核面积与周长之比  $R$  三组形态学参数,如图 3 所示。由不同种类的白细胞散点图分布可知:淋巴细胞面积最小,单核细胞面积较大且外形不规则,三种粒细胞的细胞核形状不规则。这些形态学特征在明场成像结果中有同样的体现,如图 2 所示。

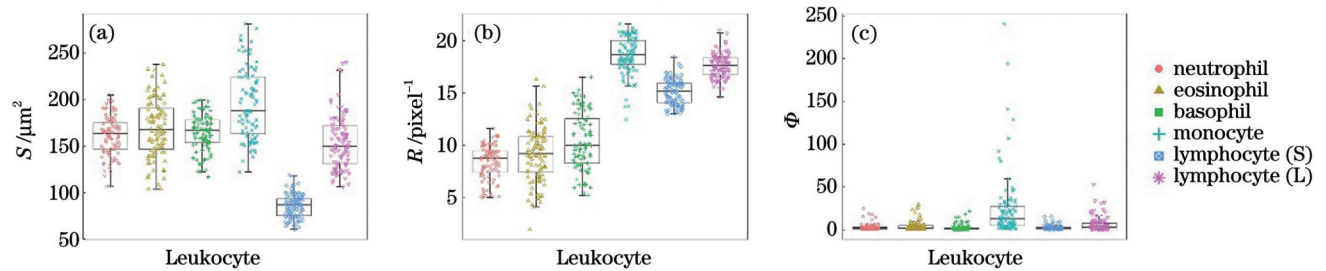


图 3 不同白细胞的三种形态学特征参数统计分析。(a)  $S$ ; (b)  $R$ ; (c)  $\Phi$   
Fig. 3 Statistical analysis of three morphological feature parameters of different leukocytes. (a)  $S$ ; (b)  $R$ ; (c)  $\Phi$

进一步对比不同白细胞及其亚结构的相位特征,得到细胞相位峰谷差  $\Delta\varphi_{\max}$ 、核质平均相位差  $\Delta\varphi_{\text{mean}}$ 、核

质相位不匹配度  $D$  三个特征参数的统计分布,如图 4 所示。图 4(a)表明,中性粒细胞和小淋巴细胞中胞核

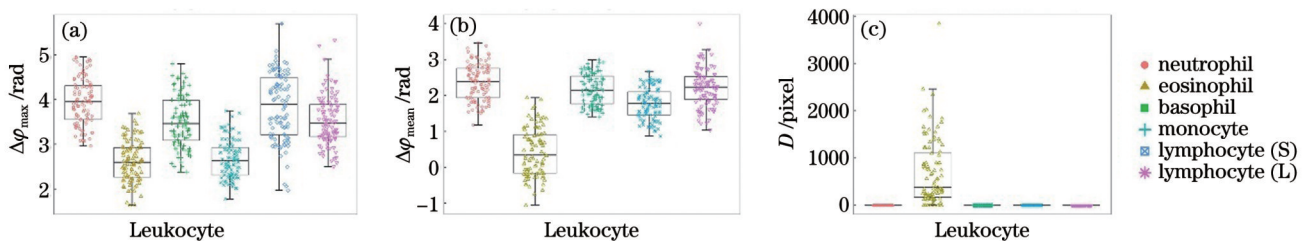


图 4 不同白细胞及其亚结构的相位特征参数统计分析。(a)  $\Delta\varphi_{\max}$ ; (b)  $\Delta\varphi_{\text{mean}}$ ; (c)  $D$   
Fig. 4 Statistical analysis of phase feature parameters of different leukocytes and their substructures. (a)  $\Delta\varphi_{\max}$ ; (b)  $\Delta\varphi_{\text{mean}}$ ; (c)  $D$

与胞质的相位差较大,而单核细胞中胞核与胞质的相位差较小。这主要是因为中性粒细胞和小淋巴细胞中胞核的染色质通常呈凝结状,且小淋巴细胞中的胞质基本无颗粒,而单核细胞中胞核的染色质疏松。由图 4(b)、(c)可知,除嗜酸性粒细胞以外,其余白细胞中胞核的平均相位均大于胞质的平均相位,嗜酸性粒细胞中细胞核与细胞质的平均相位非常接近。这是由于嗜酸性粒细胞的胞质含有大量体积大、折射率高且分布均匀的嗜酸性颗粒,故胞核与胞质的相位差异不明显<sup>[24]</sup>,这一结论也在图 2(b)中有所体现。

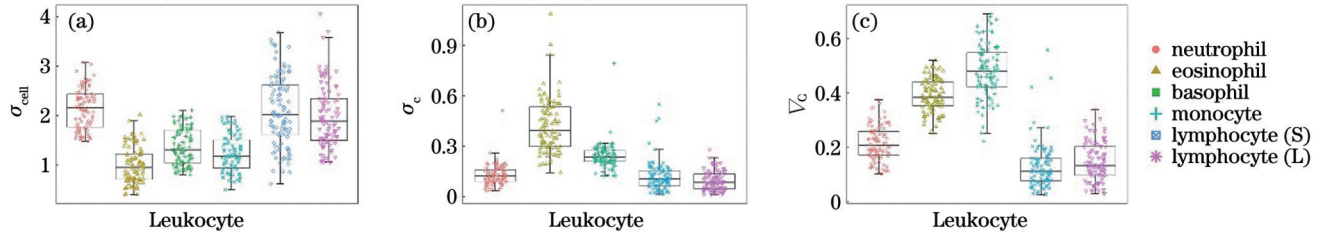


图 5 不同白细胞的相位波动特征参数统计分析。(a)  $\sigma_{\text{cell}}$ ; (b)  $\sigma_c$ ; (c)  $\nabla_c$

Fig. 5 Statistical analysis of phase fluctuation feature parameters of different leukocytes. (a)  $\sigma_{\text{cell}}$ ; (b)  $\sigma_c$ ; (c)  $\nabla_c$

上述统计结果及对比分析表明,基于明场共定位成像的白细胞及其亚结构的相位特征参数可用来区分不同种类的白细胞,辅助白细胞分类。

### 3.3 基于相位特征参数的白细胞分类

基于上述分析讨论的相位特征参数,对白细胞中依靠整体相位信息难以区分的三种粒细胞进行分类。根据前文分析,三种粒细胞的差异主要体现在核质相位不匹配度  $D$ 、细胞质方差  $\sigma_c$ 、核质平均相位差  $\Delta\varphi_{\text{mean}}$ 、细胞质方差  $\sigma_c$  和细胞质梯度分布  $\nabla_c$  四个参数上。t-SNE (t-distributed stochastic neighbor embedding) 是一种特征提取和高维数据可视化的方法<sup>[25]</sup>,由于涉及多个参数,我们利用 t-SNE 算法将相关的 4 个特征参数降至二维,得到的结果如图 6 所示,其中 t-SNE 1 和 t-SNE 2 为降维后的二维空间特征。三种粒细胞之间的重叠区域较小,说明上述 4 个特征参数对三种粒细胞有较好的区分度。在此基础上,对 300 个不同粒细

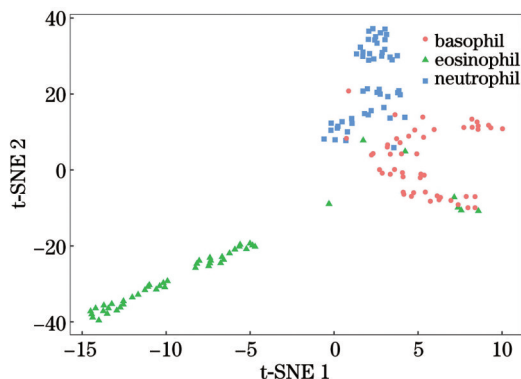


图 6 t-SNE 对三种粒细胞特征的二维表示

Fig. 6 Two-dimension representation of features of three types of granulocytes by t-SNE

为进一步区分三种粒细胞,对比研究了不同白细胞及其亚结构的相位波动特征,结果如图 5 所示。发现中性粒细胞和淋巴细胞的相位方差较大,如图 5(a)所示;而嗜酸性粒细胞中细胞质的相位方差较大,如图 5(b)所示,这同样是由于嗜酸性粒细胞的胞质中存在高折射率的嗜酸性颗粒,这也印证了之前的结论。嗜碱性粒细胞的胞质梯度较其他白细胞明显偏高,如图 5(c)所示。这主要是由于嗜碱性粒细胞的胞质含有分布不均匀且粗大的嗜碱性颗粒,故其细胞质相位波动幅度大且频繁。

胞样品(中性粒细胞、嗜酸性粒细胞、嗜碱性粒细胞各 100 个)进行分类,准确率达 94%。

### 3.4 相位特征参数辅助白细胞分类

基于明场共定位成像的相位特征参数除了可用于区分不同种类的白细胞外,还可作为附加参考信息辅助常规镜检,由于增加了额外的相位信息,可有效辅助镜检时易混淆细胞的分类:三种粒细胞的相位特征参数主要为核质相位不匹配度  $D$ 、细胞质方差  $\sigma_c$ 、核质平均相位差  $\Delta\varphi_{\text{mean}}$ 、细胞质方差  $\sigma_c$  和细胞质梯度分布  $\nabla_c$ ; 淋巴细胞与单核细胞的相位特征参数主要为相位峰谷差  $\Delta\varphi_{\text{max}}$  和核质平均相位差  $\Delta\varphi_{\text{mean}}$ ,其余特征参数为辅。

为进一步阐明该方法的有效性,针对 1200 个中性粒细胞和嗜酸性粒细胞,我们分别对比了 40 倍镜下的相位特征识别、40 倍镜下的深度学习识别和 100 倍镜下的商用血细胞分析仪 Morpho 的分类效果。其中,40 倍镜下的深度学习采用的是轻量级卷积神经网络 MobileNet<sup>[26]</sup>,100 倍镜下的商用血细胞分析仪 Morpho 对白细胞的分类准确率达 97%<sup>[27]</sup>,已被应用于各大医院。两种深度学习算法针对易混淆细胞(这里取最高预测概率低于 70%)给出不同细胞类型的可能性占比,对于图 7 所示的细胞 1 和细胞 2,40 倍镜和 Morpho 系统对易混淆细胞的预测概率分别如图 7(a3)、(b3)、(a5)、(b5)所示。作为对比,在共定位成像系统对易混淆细胞进行成像并提取其相关相位特征,得到的结果如表 2 所示。表 3 为数据统计得到的中性粒细胞和嗜酸性粒细胞的特征参数值的上下四分位数。可以看出,这 4 个相位特征值均与嗜酸性粒细胞的相位特征值吻合,而与中性粒细胞有较大

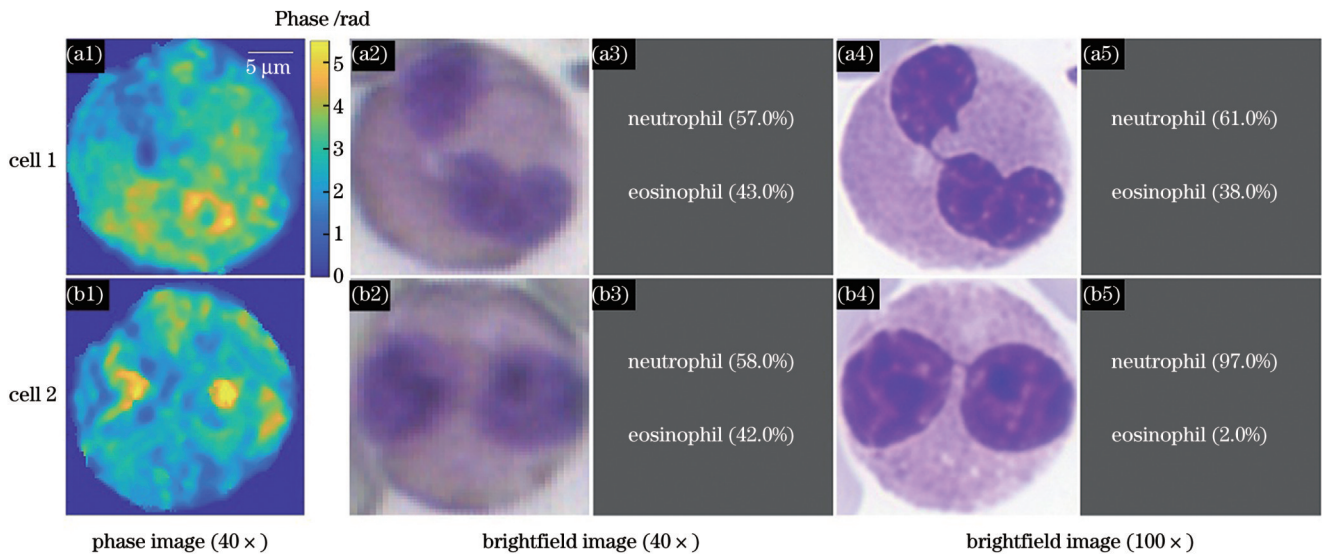


图7 易混淆细胞对比。(a1)(b1)相位图;(a2)(b2)40倍镜下的明场图;(a3)(b3)深度学习对图7(a2)、(b2)的分类准确率;(a4)(b4)100倍镜下的明场图;(a5)(b5)Morphogo系统对图7(a4)、(b4)的分类准确率  
 Fig. 7 Comparison of easily confused cells. (a1)(b1) Phase image; (a2)(b2) brightfield images under 40× object lens; (a3)(b3) classification accuracies for Figs.7 (a2) and (b2) by deep learning; (a4)(b4) brightfield images under 100× object lens; (a5)(b5) classification accuracies for Figs. 7 (a4) and (b4) by Morphogo system

表2 易混淆细胞的特征参数值  
 Table 2 Feature parameters of easily confused cells

Feature parameter	Cell 1	Cell 2
$D$ /pixel	167	1833
$\sigma_c$	0.60	0.32
$\Delta\varphi_{\text{mean}}$ /rad	0.88	0.16
$\nabla_c$	0.42	0.46

表3 中性粒细胞和嗜酸性粒细胞特征值的上下四分位数  
 Table 3 Upper and lower quartiles of characteristic values of neutrophils and eosinophils

Feature parameter	Neutrophil	Eosinophil
$D$ /pixel	(0, 0)	(172.5, 1106.0)
$\sigma_c$	(0.09, 0.16)	(0.30, 0.53)
$\Delta\varphi_{\text{mean}}$ /rad	(2.06, 2.83)	(-0.15, 0.95)
$\nabla_c$	(0.17, 0.26)	(0.35, 0.44)

差异。最后利用白细胞分类金标准——专业人工镜检方法对易混淆细胞进行类别鉴定,三位持有检验医师资格证的专业检测人员一致判定易混淆细胞均为嗜酸性粒细胞,结果与易混淆细胞的相位特征参数判定结果一致,表明相位特征参数可有效辅助白细胞分类。

### 3.5 异型淋巴细胞的定量相位分析

基于前文提出的相位特征参数,进一步研究了一种形态变异的淋巴细胞(异型淋巴细胞,又称反应性淋巴细胞)。异型淋巴细胞在健康人体外周血中的含量(细胞个数占比小于2%)极低,当人体患有病毒感染、

原虫感染、过敏等疾病后,其含量会急剧升高,是一项重要的血液检测指标。而异型淋巴细胞在人工镜检中极易与正常淋巴细胞或单核细胞混淆,容易造成误诊、误判<sup>[28]</sup>。

异型淋巴细胞的变异主要体现在细胞质含有较多的嗜碱性颗粒,细胞质整体呈蓝色,且常含有空泡。异型淋巴细胞、正常淋巴细胞的明场图像与原位相位分布如图8所示。对比异型淋巴细胞[lymphocyte(A)]与正常淋巴细胞中的平均相位差分布,如图9所示,发现异型淋巴细胞的细胞核与细胞质的平均相位差明显低于正常淋巴细胞(这里忽略了小淋巴细胞,因为其胞

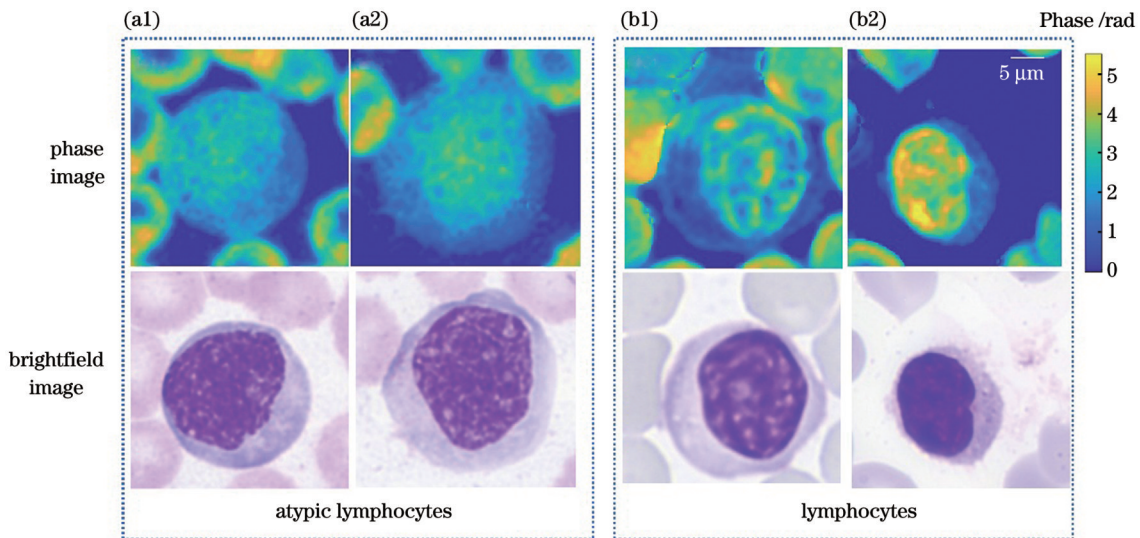


图 8 异型淋巴细胞和正常淋巴细胞的相位图及其明场图。(a1)(a2)异型淋巴细胞;(b1)(b2)淋巴细胞

Fig. 8 Phase images of atypical lymphocytes and normal lymphocytes and corresponding brightfield images. (a1)(a2) Atypical lymphocytes; (b1)(b2) normal lymphocytes

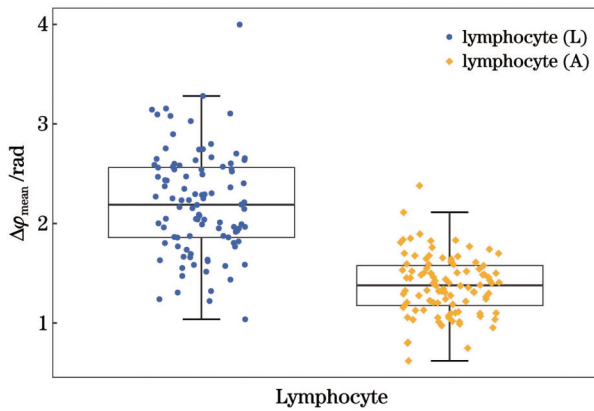


图 9 大淋巴细胞和异型淋巴细胞的相位特征

Fig. 9 Phase characteristics of large lymphocyte and atypical lymphocyte

质含量极低,比较容易区分)。利用这一相位特征对 200 个样品(异型淋巴细胞和正常淋巴细胞各 100 个)进行分类,准确率达到 84.5%。

## 4 结 论

提出了一种基于定量相位成像实现白细胞分类的方法。利用基于 DHM 和明场显微成像的共定位系统分析不同种类的白细胞,先由明场图像获得细胞的位置结构信息,提取细胞原位相位信息,再通过统计分析提取出多个有效辅助白细胞分类的特征参数,最终实现白细胞的分类。基于提取的白细胞亚结构相位特征参数对三种粒细胞进行分类,准确率达 94%。进一步分析镜检中容易混淆的异型淋巴细胞,对异型淋巴细胞的识别准确率达 84.5%。与仅使用相位信息对白细胞分类的方法相比,所提方法可将明场图像中细胞核与细胞质的高对比度引入细胞相位分布,从而有效对比不同白细胞亚结构的特征,提高分类的准确率。相

比常规镜下的检查分类,所提方法提供了额外的相位信息,可有效辅助白细胞分类。所提方法无须对常规染色血涂片样品进行特殊处理,且系统容易与显微镜集成,有望广泛用于白细胞分类、各类血液疾病的诊断和治疗。

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## Study on White Blood Cell Substructure Feature Parameters Based on Co-localized Phase Imaging

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### Abstract

**Objective** The accurate classification of white blood cells (WBCs) is crucial in the examination of blood and the diagnosis and treatment of clinical conditions. Manual examination under a bright-field microscope, the gold standard for blood cell analysis, is time-consuming and inspector-dependent. Currently, blood cell analyzers based on the impedance method or flow cytometry are extensively employed. However, some false positives may occur because of the structural variability of WBCs, which requires a manual microscopic review. In addition, these instruments are expensive. Deep learning, which can reduce the technical requirements of inspectors, is widely used for WBC classification. However, this analysis continues to rely on the morphology and color characteristics of the stained cells. To achieve high accuracy in the classification of WBCs, the process usually requires image acquisition and processing under a 100× objective lens, which can be time-consuming and data-intensive. Quantitative-phase imaging (QPI) is an effective method for studying cell morphology and biochemistry. However, identifying WBCs solely based on their phase characteristics is challenging, particularly when these phase characteristics are not prominent. Research on stained cells using QPI has shown that the inclusion of phase information, alongside bright-field pictures, might provide useful insights for WBC classification. In this study, the phase distributions of five different types of WBCs were quantitatively analyzed, and the substructure phase



information was effectively divided using a co-localization imaging system based on digital holographic microscopy (DHM) and bright-field microscopy. A series of feature parameters were extracted to assist with the WBC classification. The accuracies of the classification of the three types of granulocytes based on the extracted phase feature parameters were 94%. Additionally, atypical lymphocytes were studied, and a recognition accuracy of 84.5% was achieved. The proposed method utilizes routine blood smear samples stained for clinical microscopy, making it easy to integrate into a commercial microscopic system and providing a wide range of practical applications.

**Methods** A benchtop co-localization imaging system was used to obtain bright-field images and quantitative phase images of WBCs from peripheral blood smears of healthy individuals. Quantitative phase images of the WBCs were reconstructed from off-axis holograms obtained from DHM. To segment the phase information, WBCs were first extracted and divided into two parts, the nucleus and the cytoplasm, based on bright-field images. Then, the position information of the nucleus and cytoplasm of the WBCs in the bright-field images was transposed onto the corresponding phase images. Finally, the quantitative phase distributions of WBCs and their corresponding nuclei and cytoplasm were successfully acquired. A substantial number of WBC samples consisting of 100 neutrophils, eosinophils, basophils, monocytes, large lymphocytes, and small lymphocytes were selected for co-localization imaging and statistical analysis. Various feature parameters were extracted to quantitatively describe and analyze the morphological and substructural features of the different WBCs.

**Results and Discussions** The feature parameters of the five types of WBCs were subjected to analysis and comparison, revealing distinct phase characteristics for each type. Neutrophils had a substantially higher nuclear phase value than the cytoplasmic phase value [Fig. 4(a)], whereas eosinophils had comparable nuclear and cytoplasmic phase values (Fig. 4). The cytoplasmic phase values in basophils fluctuated substantially [Fig. 5(c)], and monocytes showed a smaller phase difference between the nucleus and cytoplasm than lymphocytes [Fig. 4(b)]. Using the extracted feature parameters, three types of granulocytes were successfully classified with 94% accuracy. The efficiency of classifying phase features was evaluated by analyzing a total of 1200 neutrophils and eosinophils. This analysis was conducted using a phase feature method based on a 40 $\times$  co-localization microscope, deep learning classification based on a 40 $\times$  brightfield microscope, and a commercial system called Morphogo with a 100 $\times$  microscope. The results showed that the phase feature accurately identified easily confused cells in deep learning classification or the Morphogo system (Fig. 7). Furthermore, an examination of atypical cells was conducted, revealing that the use of phase characteristics resulted in a classification accuracy of 84.5%. These results demonstrate that the phase feature parameters are effective in aiding WBC classification.

**Conclusions** This study proposes a method for classifying WBCs using QPI. The approach involved analyzing different types of WBCs using a co-localization imaging system that combines DHM and bright-field microscopy. The position and structural information of WBCs were obtained from bright-field images, and the phase information of WBCs and their nuclei and cytoplasm were extracted accordingly. Statistical analysis was then used to extract feature parameters that effectively aided in the classification of WBCs. This method achieved an accuracy rate of 94% for classifying the three types of granulocytes based on the substructure phase characteristic parameters. Further analysis showed an accuracy rate of 84.5% for identifying atypical lymphocytes, which are often misinterpreted during microscopic examinations. Compared with using only phase information to classify WBCs, the proposed method incorporates high contrast between the nucleus and cytoplasm in bright-field images to effectively compare the characteristics of different WBC substructures, leading to an improved classification scope and accuracy. In addition, compared to conventional microscopic classification, the proposed method provides additional phase information that can assist in WBC classification. This method is easy to integrate with microscope and does not require the special treatment of conventionally stained blood smear samples. It is expected to be widely used for the leukocyte classification and diagnosis and treatment of various blood diseases.

**Key words** bio-optics; quantitative phase imaging; digital holography; white blood cell substructures; phase distribution characteristics