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เรียน รองคณบดีฝ่ายวิจัยและบริการวิชาการ ผ่านหัวหน้าภาควิชาวิศวกรรมไฟฟ้าฯ

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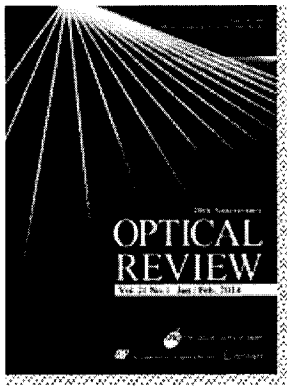

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2. Dr. Toshihiro Sakurai	10%	• Running experiment • Discussing results
3. Dr. Kazuya Iinaga	5%	• Discussing results
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REGULAR PAPER

Practical technique to quantify small, dense low-density lipoprotein cholesterol using dynamic light scattering

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Abstract Quantification of small, dense low-density lipoprotein (sdLDL) cholesterol is clinically significant. We propose a practical technique to estimate the amount of sdLDL cholesterol using dynamic light scattering (DLS). An analytical solution in a closed form has newly been obtained to estimate the weight fraction of one species of scatterers in the DLS measurement of two species of scatterers. Using this solution, we can quantify the sdLDL cholesterol amount from the amounts of the low-density lipoprotein cholesterol and the high-density lipoprotein (HDL) cholesterol, which are commonly obtained through clinical tests. The accuracy of the proposed technique was confirmed experimentally using latex spheres with known size distributions. The applicability of the proposed technique was examined using samples of human blood serum. The possibility of estimating the sdLDL amount using the HDL data was demonstrated. These results suggest that the quantitative estimation of sdLDL amounts using DLS is feasible for point-of-care testing in clinical practice.

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1 Introduction

The amounts of low density lipoprotein (LDL) cholesterol and high-density lipoprotein (HDL) cholesterol are important indicators for the diagnosis of various diseases. Moreover, the amount of small, dense LDL (sdLDL) cholesterol in LDL cholesterol is clearly associated with an increased risk of coronary artery diseases [1–4]. The size and density of sdLDL are loser to the so-called better cholesterol, or HDL, but sdLDL is actually worse for health than the large and buoyant LDL (lbLDL). Thus, there is a strong demand to quantify sdLDL levels [5–7]; however, this is not an easy task. The currently available sdLDL measurement process is complicated and time consuming. On the other hand, the amounts of LDL cholesterol and HDL cholesterol are commonly measured in chemical assays; these values are available through a routine medical check or a blood test. In the lipoprotein measurement, the LDL (sdLDL + lbLDL) or HDL + sdLDL component can be separated from the blood serum as a pretreatment for the blood test. Thus, if the ratio of one species to the total amount can be determined, the sdLDL amount can be obtained using the commonly available LDL and HDL values.

Several methods such as high-performance gel-filtration chromatography [8], ultracentrifugation [9], nuclear magnetic resonance [10], and electrophoresis [11, 12] are available to quantify the amount of one species from the others. However, these methods are complicated and laborious. Hence they are not suitable for the point-of-care testing (POCT) in clinical practice. Recently, the chemical assay for sdLDL measurement has become commercially

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available [13]; however, it is still not commonly used in clinical measurement, which needs special equipment.

Physical assay such as particle sizing generally requires less time and seems promising for POCT. Particle counters such as the Coulter counter and the cell-sorter are well-known methods for particle sizing [14]. However, they require a special technique for constructing a rapid flow as fine as the size of a particle. This requires large and complicated equipment. An optical scattering technique provides an alternative approach for performing the physical assay. The size distribution of particles suspended in liquid can be obtained using the dynamic light scattering (DLS) principle. Previous studies have reported the use of DLS for measuring the sizes of lipoproteins such as HDL [15], LDL [16, 17], and chylomicron [18]. These techniques have been used for investigating particle sizes or size distributions. They can provide information on particle sizes or the proportion of particle amounts. However, there have been few reports that show the optical scattering technique to estimate the amount of the small dense LDL from the LDL or HDL values. Although the sdLDL amount may be quantified from the size distribution obtained in these DLS measurements, the estimated size distributions are known to be neither stable nor repeatable for bimodal or multimodal distributions [18–20]. Various attempts to improve the data analysis methods have been reported [19–22]. Unfortunately, there is still no general method widely used for estimating the fraction of one species out of several others.

In our previous work [20], we derived a solution to obtain the weight fraction of a species in the DLS measurement. In the derivation, we assumed unnatural size distributions such as a delta function and a rectangular function. The solution included an infinite series expansion of a gamma function. In this work, we have newly derived an analytical solution for the Gaussian distribution that is more natural for biological particles such as lipoproteins. It is in a closed form and is more useful in practice than the previous solution. Using this technique, the sdLDL amount can be quantified from the LDL or HDL data. Since a fast measurement of LDL and HDL is common, we can expect to realize the POCT for sdLDL as well.

2 Methods

2.1 Principle of fraction estimation

When a sample solution comprises a suspending medium and scattering particles with a size distribution, the normalized first-order autocorrelation function measured in DLS is given as [18]

$$g^{(1)}(\tau) = \int_0^\infty f(r) \exp\left(-\frac{\alpha\tau}{r}\right) \mathrm{d}r, \tag{1}$$

where τ is the correlation time, $\alpha = q^2kT/3\pi\eta$, q is the scattering vector given by $q = (4\pi n/\lambda)\sin(\theta/2)$, and n, λ, θ, k, T, and η are the refractive index of the medium, the wavelength of light, the scattering angle, the Boltzmann constant, the absolute temperature, and the viscosity of the medium, respectively. $f(r)$ is the normalized intensity size distribution and r is the particle size. We can obtain $f(r)$ from the measured $g^{(1)}(\tau)$ by solving Eq. (1) with conventional techniques. Once $f(r)$ is obtained, it is easy to determine the fraction of sdLDL. However, this process requires integral inversion and as mentioned above, the solution $f(r)$ obtained in this way is often unstable and nonrepeatable in the bimodal case [18–20]. Therefore, we propose an analytical solution for directly estimating the fraction of a given species via the autocorrelation functions.

In the DLS measurement, we measure the second-order autocorrelation function $g^{(2)}(\tau)$ of the scattered intensity, and $g^{(1)}(\tau)$ is obtained using the Siegert relation as

$$g^{(1)}(\tau) = \sqrt{\frac{g^{(2)}(\tau) - 1}{c}}, \tag{2}$$

where c is the instrumental parameter ($0 < c < 1$) related to instrument setting.

When a sample solution comprises two kinds of scatterers with different size distributions, the total autocorrelation function, $g_{\mathrm{sl}}^{(1)}(\tau)$ is given as [21, 22]

$$g_{\mathrm{sl}}^{(1)}(\tau) = \frac{I_{\mathrm{s}} g_{\mathrm{s}}^{(1)}(\tau) + I_{\mathrm{l}} g_{\mathrm{l}}^{(1)}(\tau)}{I_{\mathrm{s}} + I_{\mathrm{l}}}, \tag{3}$$

where I is the intensity-weight, and subscripts s and l refer to the small and large particle sizes or

$$I_{\mathrm{s}} = \int_0^\infty f_{\mathrm{s}}(r)\mathrm{d}r, \quad I_{\mathrm{l}} = \int_0^\infty f_{\mathrm{l}}(r)\mathrm{d}r. \tag{4}$$

If we define the intensity fraction of the small particles as $X_{\mathrm{I}} = I_{\mathrm{s}}/(I_{\mathrm{s}} + I_{\mathrm{l}})$, Eq. (3) reduces to

$$g_{\mathrm{sl}}^{(1)}(\tau) = X_{\mathrm{I}} g_{\mathrm{s}}^{(1)}(\tau) + (1 - X_{\mathrm{I}}) g_{l}^{(1)}(\tau). \tag{5}$$

Equation (5) can be solved for the fraction X_I as

$$X_{\mathrm{I}} = \frac{g_{\mathrm{sl}}^{(1)} - g_{\mathrm{l}}^{(1)}}{g_{\mathrm{s}}^{(1)} - g_{\mathrm{l}}^{(1)}}. \tag{6}$$

In Eq. (6), the uncertainty of the instrumentation parameter or square root of ($1/c$) in Eq. (2) is cancelled out. In this way, we can obtain the fraction from the measured $g_{\mathrm{sl}}^{(1)}(\tau)$ when $g_{\mathrm{s}}^{(1)}(\tau)$ and $g_{\mathrm{l}}^{(1)}(\tau)$ are available beforehand. A similar method was proposed in our previous work, in which the size distribution of scatterers was assumed to be

either a delta function or a rectangular function [20]. With natural biological particles such as lipoproteins, the Gaussian size distribution is more appropriate. When the mean m and the SD σ of the particle size are available, we can assume the size distribution of scatterers as

$$f(r) = \frac{1}{\sqrt{2\pi}\sigma}\exp\left[-\frac{(r-m)^2}{2\sigma^2}\right]. \tag{7}$$

Then, the autocorrelation function of each species is

$$g^{(1)}(\tau) = \frac{1}{\sqrt{2\pi}\sigma}\int_0^\infty \exp\left[-\frac{(r-m)^2}{2\sigma^2} - \frac{\alpha\tau}{r}\right]\mathrm{d}r. \tag{8}$$

To perform the integration, we apply the following approximation using the Taylor expansion at $r = m$,

$$\exp\left(-\frac{\alpha\tau}{r}\right) \approx \exp\left(\frac{\alpha r\tau}{m^2} - \frac{2\alpha\tau}{m}\right). \tag{9}$$

Since Eq. (9) is 0 for a negative value of r, we can rewrite Eq. (8) using Eq. (9) as shown below,

$$g^{(1)}(\tau) \approx \frac{1}{\sqrt{2\pi}\sigma}\exp\left(-\frac{2\alpha\tau}{m}\right) \times \int_{-\infty}^{\infty} \exp\left[-\left(\frac{r-m}{\sqrt{2}\sigma}\right)^2 + \frac{\alpha r\tau}{m^2}\right]\mathrm{d}r. \tag{10}$$

In this form, the integral can be calculated as

$$g^{(1)}(\tau) \approx \exp\left[\left(\frac{\sigma\alpha\tau}{\sqrt{2}m^2}\right)^2 - \frac{\alpha\tau}{m}\right]. \tag{11}$$

Then, the fraction of the scattered intensity can be estimated as

$$X_\mathrm{I} = \frac{g_\mathrm{sl}^{(1)}(\tau) - \exp\left[\left(\frac{\sigma_\mathrm{l}\alpha\tau}{\sqrt{2}m_\mathrm{l}^2}\right)^2 - \frac{\alpha\tau}{m_\mathrm{l}}\right]}{\exp\left[\left(\frac{\sigma_\mathrm{s}\alpha\tau}{\sqrt{2}m_\mathrm{s}^2}\right)^2 - \frac{\alpha\tau}{m_\mathrm{s}}\right] - \exp\left[\left(\frac{\sigma_\mathrm{l}\alpha\tau}{\sqrt{2}m_\mathrm{l}^2}\right)^2 - \frac{\alpha\tau}{m_\mathrm{l}}\right]}. \tag{12}$$

where m_s, m_l, σ_l and σ_s are the means and the SDs of the size distribution with the subscripts indicating small and large particles.

Therefore, we can obtain the fraction of one species of scatterers from the measured autocorrelation function with the mean and the SD of each species that are commonly available beforehand. This solution is in a closed form, and does not include the calculation of infinite series expansion of a special function, as in our previous work [20].

The weight fraction is commonly used in clinical applications. We defined the weight fraction of the small particles as $X_\mathrm{W} = W_\mathrm{s}/(W_\mathrm{s} + W_\mathrm{l})$, where W is the weight of each species. The weight fraction X_W is obtained from the intensity fraction X_I using the light scattering characteristics of the scatterers. The size of lipoproteins is much smaller than the wavelength of the probing light in DLS measurement. They are known to be nearly spherical [23] and are distributed randomly in position and orientation in DLS measurement. The effect of multiple scattering is neglected when we dilute the sample solution sufficiently in DLS measurement. Therefore, we can consider this scattering condition as Rayleigh scattering. Since the scattered intensity of Rayleigh scattering is proportional to the square of the particle's volume, the total autocorrelation function for size-distributed scatters can be written as

$$g^{(1)}(\tau) = \frac{\int_0^\infty i(r)g^{(1)}(\tau,r)\mathrm{d}r}{\int_0^\infty i(r)\mathrm{d}r} = \frac{\int_0^\infty w(r)r^3g^{(1)}(\tau,r)\mathrm{d}r}{\int_0^\infty w(r)r^3\mathrm{d}r} = \frac{\int_0^\infty n(r)r^6g^{(1)}(\tau,r)\mathrm{d}r}{\int_0^\infty n(r)r^6\mathrm{d}r}, \tag{13}$$

where $g^{(1)}(\tau, r)$ is the autocorrelation function of scatterers of a specific size r, and $i(r)$, $w(r)$, and $n(r)$, respectively, signify size distributions of scattered intensity, scatterers' weight and scatterers' number. Consequently, X_W can be estimated from X_I as

$$X_\mathrm{W} = \frac{X_\mathrm{I}m_\mathrm{l}^3}{X_\mathrm{I}(m_\mathrm{l}^3 - m_\mathrm{s}^3) + m_\mathrm{s}^3}. \tag{14}$$

It should be noted that we can obtain the fraction analytically as a single number using Eqs. (12) and (14). This is a significant advantage of this technique over the common parameter-fitting techniques. They usually require the iteration processes, and inevitably have the problem of converging in a local minimum.

In a common blood test, the amounts of LDL and HDL are available. The size range of the sdLDL is inside and outside of the LDL and the HDL size distributions, respectively. Therefore, using the weight fraction X_W we can quantify the sdLDL amount with the measured amount of LDL or HDL in the following two ways: either by sdLDL = LDL × X_W or by sdLDL = HDL × $(1 - X_\mathrm{W})/X_\mathrm{W}$.

2.2 Accuracy of proposed principle

In the above derivation, we used the approximation of Eq. (9). To examine the applicability of the proposed technique to the practical range of sdLDL amounts, we checked the accuracy of this approximation. The appropriateness of the approximation was evaluated in terms of the correlation coefficient between the functions $g^{(1)}(\tau)$'s obtained from Eqs. (8) and (11) for different particle sizes. The $g^{(1)}(\tau)$ of Eq. (8) was obtained by the numerical calculation of the integral.

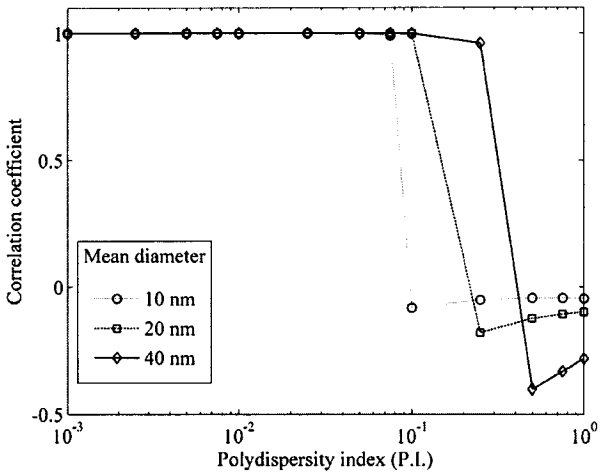

Fig. 1 Correlation between the autocorrelation functions obtained from Eqs. (8) and (11)

The result is shown in Fig. 1. The abscissa is the polydispersity index (P.I.) defined as $(\sigma/m)^2$ where σ and m are the SD and the mean of the Gaussian size distribution [24]. The mean diameters were set as 10.0, 20.0, and 40.0 nm, which are typical sizes of HDL, LDL, and VLDL, respectively. The result shows that the approximation of Eq. (11) holds well in the range of P.I. from 0.001–0.07. Therefore, this analysis confirms that we can use Eq. (12) for the practical range of LDL and HDL, or P.I. = 0.001–0.06.

Since Eq. (14) is a function of the cubes of the mean sizes, strong nonlinear characteristics are expected in the conversion from the intensity fraction X_I to the weight fraction X_W. Thus, we examined the sensitivity of the conversion for the different size ratio between two species of the scatterers. Figure 2 shows the dependence of X_W on X_I. As

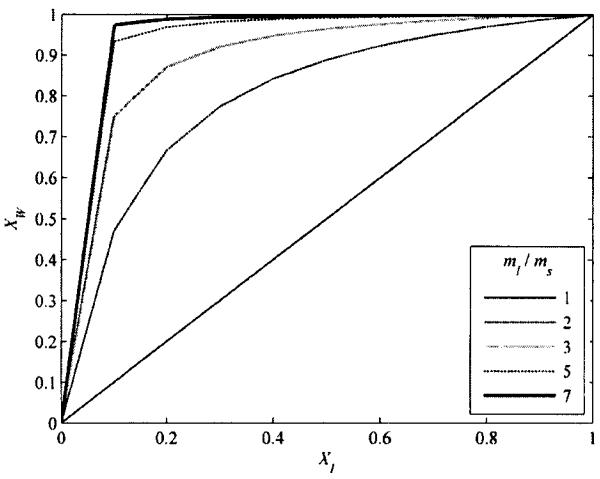

Fig. 2 Effect of the particle size ratio on the conversion from the intensity fraction to the weight fraction

the size ratio m_l/m_s becomes large, the conversion curve rises and then saturates at a small value of X_I. This analysis shows that the conversion given by Eq. (14) should be used for a size ratio less than 3. Particle size of lipoproteins has been evaluated using several methods. According to the Ref. [25], the particle size ranges 7.3–13, 18.0–21.2 and 21.2–23.0 nm for HDL, sdLDL and lbLDL, respectively. Since the highest possible size ratios of lbLDL/sdLDL and sdLDL/HDL are 1.28 and 2.90, respectively, the use of Eq. (14) for the conversion is appropriate.

3 Measurement

3.1 DLS measurement

The accuracy of the proposed technique was tested using latex spheres of two different sizes with a known fraction. Then, the applicability of the proposed technique to the sdLDL quantification was examined in the experiment with samples of human blood serum. The sdLDL amount estimated by the proposed technique was compared to the values obtained in the conventional chemical assay.

The autocorrelation function $g_{sl}^{(1)}(\tau)$ of the sample solution was measured using a DLS system (FDLS-3000, Otsuka Electronics Co., Ltd.). The laser power and the wavelength were 100 mW and 532 nm, respectively. The scattered light was detected at $\theta = 90°$. The temperature was set at 37 °C. The measurements were repeated three times for each sample. The sampling and correlation times were set at 8 and 1024 μs, respectively.

3.2 Noise elimination in autocorrelation function

The method of fraction estimation using Eq. (12) is based on a calculation that uses the noise-free autocorrelation function for the monomodal case and the measured autocorrelation function for the bimodal mixture. However, the latter function inevitably includes various noises, such as the scattering from larger debris, and aggregates. To suppress the effect of this noise, we devised the following technique. We eliminated the autocorrelation component of much larger scatterers (typically several hundred nm) than LDL (<30 nm) to separate the signals of the scatterers of interest. Because of this large difference in size, the autocorrelation component of the noise scatterers could be separated. It can be approximated as an exponential function of large time constant as shown in Fig. 3. The major component in a much shorter correlation time, or the signal from the rapidly moving scatterers is the signal of interest. Therefore, we subtracted the estimated autocorrelation component of the noise scatterers from the measured autocorrelation function. This major component after the

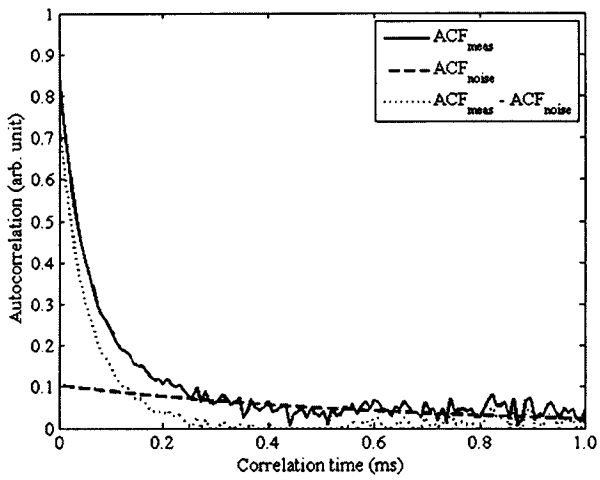

Fig. 3 Principle to eliminate contribution from noise scatterers: measured autocorrelation function (ACF_{meas}), fitted exponential component of much larger scatterers (ACF_{noise}), and data used for proposed technique ($ACF_{meas} - ACF_{noise}$)

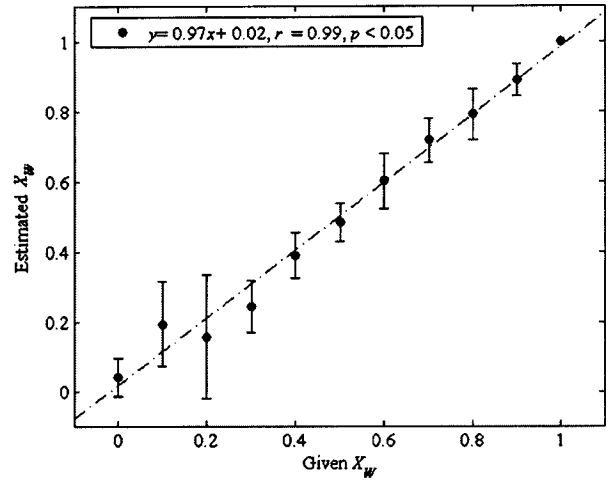

Fig. 4 Results of fraction estimation in DLS for bimodal distribution of PS particles

subtraction was then used as the measured $g_{sl}^{(1)}(\tau)$ in Eq. (12). Figure 3 illustrates this process.

4 Accuracy test with artificial scatterers

To test the accuracy of the proposed technique, an experiment was performed with the latex particles (polystyrene spheres, PS) of known sizes (diameters of 21 ± 5.7 nm and 28 ± 6.1 nm; MAGSPHERES INC.). The two-size PS particles were diluted with pure water to the same concentration. The samples were then prepared by mixing the particles in different weight fractions X_W.

A comparison between the X_W of the prepared sample and that of the estimation is shown in Fig. 4. The regression line is close to the ideal line ($y = x$) with a high correlation coefficient. This result verifies that the proposed technique is effective in estimating the weight fraction of one of the species out of two as long as the mean and SD of each species are correctly known.

5 Estimation of sdLDL from LDL

To test the applicability of the proposed technique to the quantification of sdLDL, experiments were performed with samples of human serum of 24 subjects. The LDL (sdLDL + lbLDL) was separated from human serum using the pretreatment reagent of the chemical assay (LDL-EX, Denka Seiken Co., Ltd., Japan). The separated LDL solution was then diluted with sterile 0.9 % saline to make the optical density much <1.00 and the autocorrelation function of the scattered intensity was obtained in the DLS measurement. Using the autocorrelation function, the weight fraction X_W was obtained according to Eqs. (12) and (14). According to the Ref. [25], the values of the mean and the SD for sdLDL and lbLDL were set as $m_s \pm \sigma_s = 19.8 \pm 1.5$ and $m_l \pm \sigma_l = 22.1 \pm 1.0$ nm, respectively. The LDL cholesterol concentration was measured in the chemical assay using a commercial test kit (LDL-EX, Denka Seiken Co., Ltd., Japan). We refer to this value as LDL_{meas}. The sdLDL amount was then estimated as $sdLDL_{est} = X_W \times LDL_{meas}$. For comparison, we measured the sdLDL amount in a chemical assay using a commercial assay kit (sdLDL-C, Denka Seiken Co., Ltd., Japan). We refer to this value as $sdLDL_{meas}$.

The comparison between $sdLDL_{est}$ and $sdLDL_{meas}$ is shown in Fig. 5. A positive correlation was obtained, but the correlation coefficient was very low. In some cases, the

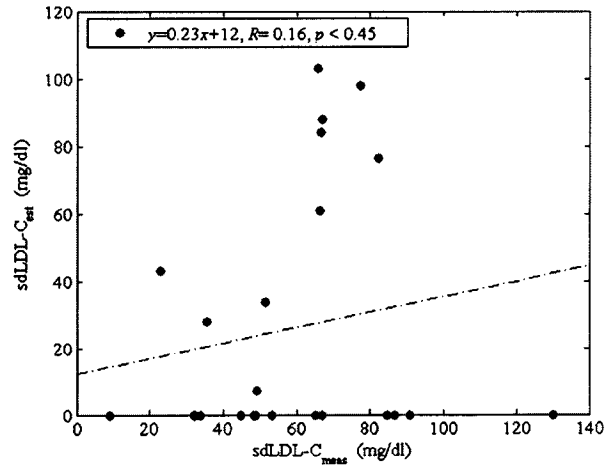

Fig. 5 Correlation between the sdLDL amount measured in chemical assay and sdLDL amount estimated from the LDL value

fraction of sdLDL was estimated as zero even when non-negligible values were measured in a chemical assay. To investigate the cause of this problem we analyzed the accuracy of X_W instead of $sdLDL_{est}$, because it includes the effect of LDL_{meas} as $sdLDL_{est} = X_W \times LDL_{meas}$. Figure 6 shows the relation between $sdLDL_{est}$ and X_W. This reveals that $sdLDL_{est}$ was zero because X_W was zero even when $sdLDL_{meas}$ was large. This occurs when the separation of size distributions for two species is insufficient, and an overlapped distribution of bimodal scatterers appears as if it were monomodal, or $g_{sl}^{(1)}(\tau) \approx g_l^{(1)}(\tau)$. As mentioned above, the typical size ranges of sdLDL and lbLDL are close as $m_s \pm \sigma_s = 19.8 \pm 1.5$ and $m_l \pm \sigma_l = 22.1 \pm 1.0$ nm, respectively. This phenomenon is more prominent when the amount of the smaller species is low, or X_W is small. The scattered intensity is then strongly dependent on the larger scattering particles, and the effect of smaller particles can be neglected. These facts made the accurate estimation of the fraction of smaller species difficult. Figure 6 shows that X_W was inaccurate particularly when $X_W < 0.1$. Figure 7 shows the relation between $sdLDL_{est}$ and $sdLDL_{meas}$ under the new rule in which we eliminate the data when $X_W < 0.1$ as an unreliable range. We can see the improvement in the correlation coefficient from 0.16 to 0.70. This result shows the limitation of the proposed technique for the estimation of the sdLDL fraction using the value of the LDL cholesterol.

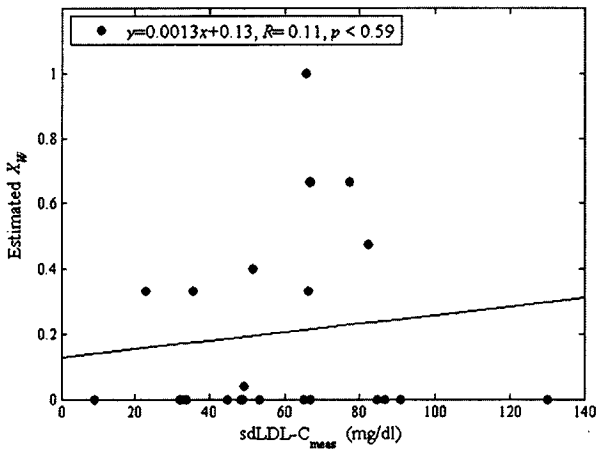

Fig. 6 Dependence of estimated fraction on measured sdLDL for analysis of low correlation in sdLDL estimation using the LDL value

6 Estimation of sdLDL from HDL

The feasibility of estimating the sdLDL amount from the measured HDL value was examined experimentally. The mixture of HDL and sdLDL was separated by applying the pretreatment reagent (sdLDL-C, Denka Seiken Co., Ltd., Japan) for the samples of human serum of 17 subjects. The fraction of the smaller species, or X_W, was obtained in the DLS measurement using the proposed technique. According to the Ref. [24], the mean and SD values for HDL and sdLDL were set as $m_s \pm \sigma_s = 10.2 \pm 2.5$ and $m_l \pm \sigma_l = 19.8 \pm 1.5$ nm, respectively. The amount of HDL cholesterol was measured in the common chemical assay with a commercial assay kit (HDL-EX, Denka Seiken Co., Ltd., Japan). We refer to this value as HDL_{meas}. The amount of sdLDL cholesterol was then estimated as $sdLDL_{est} = HDL_{meas} \times (1 - X_W)/X_W$. For comparison, we measured the amount of sdLDL cholesterol in the chemical assay using a commercial assay kit (sdLDL-EX, Denka Seiken Co., Ltd., Japan). We refer to this value as $sdLDL_{meas}$.

The comparison between $sdLDL_{est}$ and $sdLDL_{meas}$ is shown in Fig. 8. Since the separation in the size

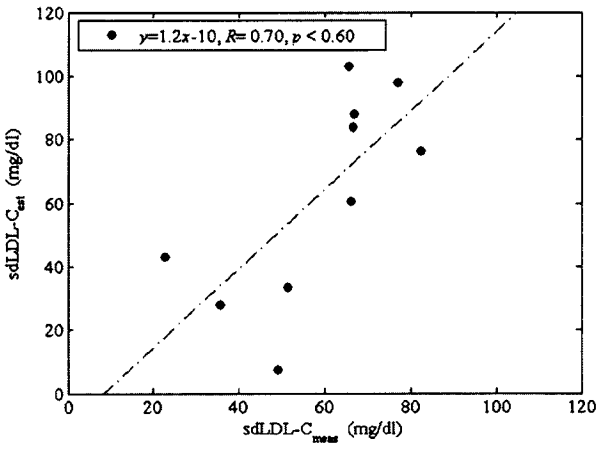

Fig. 7 Correlation between the $sdLDL_{meas}$ and $sdLDL_{est}$ with new rule to eliminate extraordinary low fraction

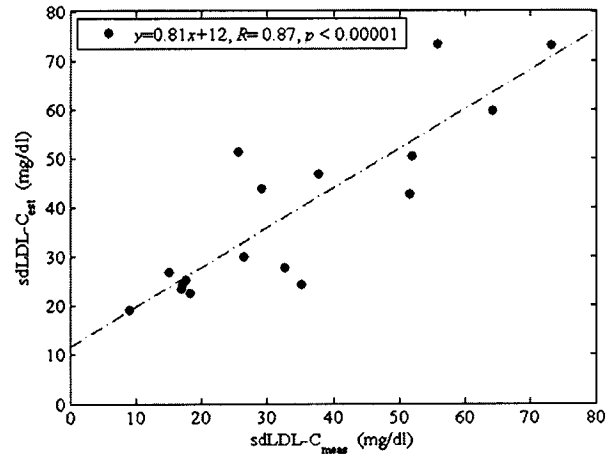

Fig. 8 Correlation between the sdLDL amount measured in the chemical assay and sdLDL amount estimated from the HDL value

distribution of two species is clear, we can see better agreement than the case of LDL. The upper shift of the regression line or the systematic overestimation can be attributed to the methodological difference between the optical and the chemical assay. $sdLDL_{meas}$ is the value from the chemical assay. It is the value of the chemical component of sdLDL and is proportional to the total volume of sdLDL in serum. On the contrary, $sdLDL_{est}$ is the value from X_W obtained from optical scattering. X_W is the ratio of small particles, or HDL in this case. The increase in $sdLDL_{meas}$ is caused by the total volume increase of sdLDL. There are two origins for this increase, or the increase in the number of sdLDL particles or of the volume of each sdLDL particle. The former increase results in the linear increase in scattered intensity, while the latter increase results in the quadratic increase in scattered intensity. Theoretically, it is taken into account as in Eqs. (13) and (14). However, in real measurements with inevitable measurement noise, the stronger slowly-varying intensity-signal tends to be emphasized more than the weaker rapidly-varying intensity-signal due to the difference in the signal to noise ratio. This results in the emphasis of ACF for larger particles. Thus, the ratio of larger particles $(1 - X_W)$ becomes more than the value of linear proportion of the chemical volume of sdLDL. According to this physical origin, $sdLDL_{est}$ in the optical measurement is over-estimated when compared to $sdLDL_{meas}$ of the chemical assay. Here, the over-estimated degree was identified as a typical example, and the calibration was made to compensate for the difference between the chemical and the optical measurements. We can correct this difference using the regression line in Fig. 8 as a calibration curve. Figure 9 shows the result after this correction. 94 % of the data are in the range of 20 mg/dl accuracy. This range is comparable to the natural variation of sdLDL among the general public [26]. This result suggests the feasibility of the optical quantification of sdLDL using the measured HDL cholesterol value with a relatively simple pretreatment process.

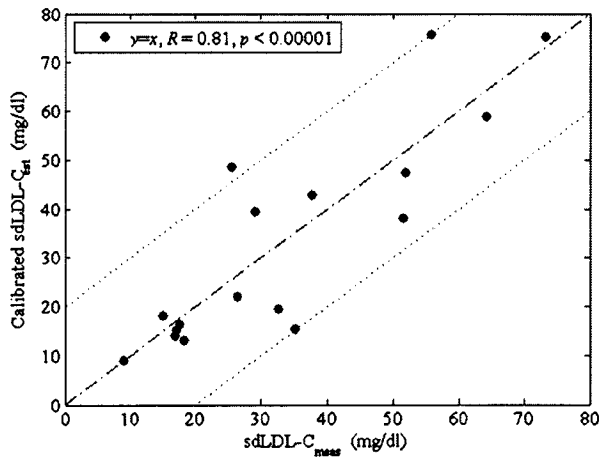

Fig. 9 Correlation between the sdLDL amount measured in the chemical assay and the sdLDL amount estimated from the HDL value with calibration

7 Conclusions

For realizing the point of care testing of sdLDL amounts, a technique to estimate the fraction of one component of scatterers using the dynamic light scattering method was developed. In this technique, we assumed the size distribution of scatterers to be the sum of two Gaussian distributions. On the basis of this assumption, we derived an analytical solution in a closed form for the fraction of one species of the scatterers. Using the values for the mean and SD of the two Gaussian distributions, the fraction of the species is calculated from the autocorrelation function of the scattered intensity obtained in the DLS measurement. Using the value of this fraction, we can estimate the amount of one species using the values for the total amount or for the amount of another species.

The accuracy of the proposed technique was confirmed experimentally using latex spheres with known size distributions. The applicability of the proposed technique to blood lipoproteins was examined experimentally using samples of human blood serum. The sdLDL amount estimated from the LDL amount was less accurate than that estimated from the HDL amount. This result showed the limitation of the proposed technique in the case when the distributions of two species are close, such as for sdLDL and lbLDL. The sdLDL amount estimated from the HDL value and calibrated for systematic deviation showed a good correlation with the amount measured in a chemical assay. Further study is required to examine the applicability of this technique to patients in clinical conditions.

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Compliance with ethical standards

Informed consent Informed consent was obtained from all the individuals participating in this study. This study was approved by the ethics review board of the Faculty of Health Sciences, Hokkaido University, Japan (approval number 09-38).

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