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Review

Resonance light scattering and derived techniques in analytical chemistry: past, present, and future

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Abstract. From 1993 to 1995, with a conventional fluorescence spectrophotometer (CFS) (convenient) and working in a synchronous scan model (easy-to-use), Pasternack et al. proposed the resonance light-scattering (RLS) technique, to efficiently characterize self-assemblies or self-aggregations of chromophores with good electronic coupling. Incident wavelengths were specially considered within their absorption envelopes (rather unorthodox), and their amplified signals were observed (good sensitivity and selectivity). Due to these absorbing benefits, RLS technique, as a novel readout method, commenced on its exciting analytical tours soon after Liu et al. and especially Li et al., separately, set out their corresponding pioneering investigations from 1995 to 1997. From then on, it has received an increasing attention by analysts, as a consequence exhibiting more and more fascinating analytical applications. Moreover, various attractive RLS-derived techniques have been developed successively to improve it or to enlarge its possibilities. Later on, Liu et al. and Li et al., Tabak et al., Yguerabide et al., Huang et al., Lakowicz et al. and Fernández Band et al. have made their outstanding contributions. In this review, we concentrate on major achievements of RLS in analytical chemistry for over a decade, involving the developments and analytical applications of RLS derived techniques treated as an impacting progress of RLS technique in analytical chemistry. Finally, an indication of future directions of RLS technique in analytical chemistry is provided.

Key words: Resonance light-scattering (RLS) technique; RLS derived techniques.

An introduction to resonance light-scattering (RLS) technique

Brief description of light scattering

The term of light scattering may not be familiar or interesting to all of us, which however, occurs in our life far and wide. Multicolored rainbows, gorgeous

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sunrise and sunset glows and a variety of colors of precious stones, for example, are all its visual manifestations. Without any exaggerations, we cannot enjoy our multihued life at all without it.

Exposed in an electromagnetic field, any system with optical heterogeneity, either resulting from the scale of a single molecule or that of self-aggregations or self-assemblies of a few or large numbers of molecules, can be observed to scatter light in all directions excluding that of an incident electromagnetic wave propagating [1, 2]. In accordance with electromagnetic theory, scattered light, treated as an secondary radiation, is radiated by electrons in scatterers of a scattering system, which are accelerated and set into oscillatory motion with the excitation of an incident electromagnetic wave [1, 2]. Since all systems, with the exception of a vacuum, are more or less heterogeneous, light scattering thus exists widely in the universe.

An overview of its establishment

In 1993, Pasternack et al. [3] was observed that by synchronously scanning both monochromators of a conventional fluorescence spectrophotometer (CFS), the light-scattering signals of *trans*-bis(*N*-methylpyridinium-4-yl)-diphenylporphine and its copper(II) derivatives, in the Soret region, all enhanced several orders of magnitude, respectively, while self-assembling on DNA. Hereby, an unorthodox resonance light-scattering (RLS) technique, focusing on observing exceptionally enhanced light-scattering signals near or within absorption envelopes of extended chromophores, was set up. In the next two years, via RLS technique Pasternak et al. probed successfully the self-aggregations of porphyrin diacid [4] and chlorophyll a [5] as well. In 1995, Pasternack and Collings [6] recommended RLS technique on *Science*.

The establishment of RLS technique, we think, is of great significance, as a result of being characterized by the following features:

(I) Good selectivity and sensitivity. Unlike regularly studied conventional Rayleigh scattering [7, 8], RLS detections just concentrate on light-scattering signals at or near absorbing regions of scattering systems. Therefore, RLS technique covers the merits of good selectivity, contributed by corresponding absorption spectra, and fine sensitivity, stemming from the factors in detailed below mentioned.

(II) Good convenience. RLS signals, augmented several orders of magnitude, can be satisfactorily measured by a CFS in contrast to relatively complicated instruments, for instance a laser source, necessary for conventional Rayleigh scattering [7, 8].

(III) Great potential in analytical chemistry. As a novel and powerful signal generation and detection platform, RLS technique is supposed to experience fresh and interesting attempts in analytical chemistry in numerous formats.

It is the three merits that urge on its introduction and wide applications in analytical events soon.

The components of its signals

At present, scales of scatterers in most RLS investigations are presumed in the Rayleigh range, so that RLS is often termed resonance Rayleigh scattering (RRS) [8, 9]. Also, because of its performance and a CFS not distinguish tiny wavelength deviations between incident and emitted beams, RLS is surely attributed to elastic light scattering, still including signals of quasi-elastic light scattering, Brillouin scattering and fluorescence [6, 11, 12]. Moreover, RLS signals are disturbed noticeably especially when ambient temperature is relatively high or comparatively wide slit-widths ($\geq$10 nm) of both monochromators are put into use [11, 12]. In conclusion, the components of RLS signals are summarized as Fig. 1.

Its major achievements in analytical chemistry

In our opinion, over a decade (from 1995 to 2006) of RLS technique applied in analytical chemistry can be separated into four stages and key achievements, in each stage are listed in brief as the following and plotted as Fig. 2 for an open-and-shut use as well.

(I) Its commencement on analytical events (1995–1997).

(II) Its theory discussion and RLS Particles^{TM} development (1998–2000).

(III) Its derived techniques, including total internal reflected resonance light scattering (TIR-RLS) and backscattering light (BSL), both proposed to probe aqueous-oil interfaces, corrected resonance light scattering (CRLS), resonance light scattering imaging (RLSI) and flow-injection resonance light scattering (FI-RLS) (2001–2004).

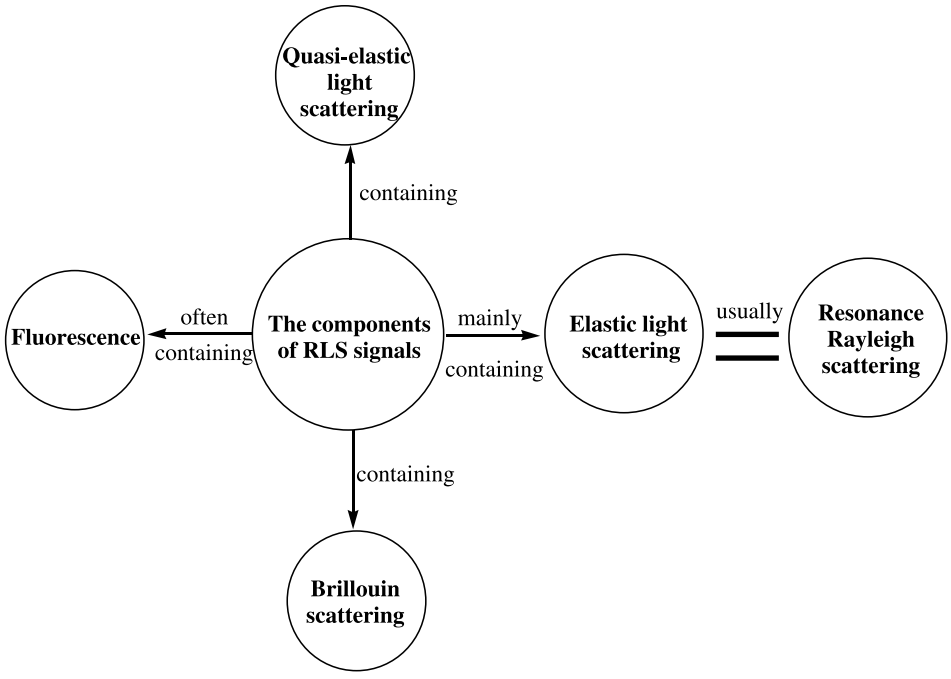

Fig. 1. The components of RLS signals

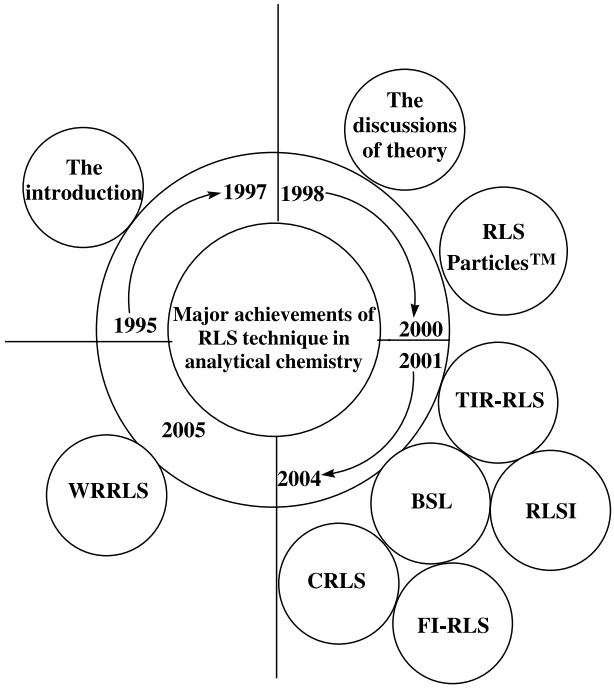

Fig. 2. Major achievements of RLS technique in analytical chemistry

(IV) Its another derived technique, i.e. wavelength-ratiometric RLS technique (WRRLS) technique (2005–2006).

In this review, we will discuss the main achievements of RLS technique in accordance with the four stages mentioned above.

The past of resonance light-scattering (RLS) technique in analytical chemistry (1995–2004)

Its commencement in analytical chemistry (1995–1997)

Prior to 1995, RLS technique is all absorbed in characterizing chromophores of self-aggregations or self-assemblies, not yet involving other purposes. However, the situation was soon changed since 1995, as Fig. 2 displays.

The contribution from Liu et al.

In 1995 and 1996, with a CFS Liu et al. proposed a resonant luminescence (RL) technique [13, 14], renamed RRS technique by them after 1997 [15–17], first to study some ion-association complexes. It was found that the enhanced RL were in direct proportion to the concentrations of investigated metal and nonmetal ions over proper concentration ranges, revealing that RL technique was a new means not only to characterize ion-association reactions but also to determine trace amounts of metal ions. Especially, the maximum RL

peaks were all found to be located between those of both absorption and fluorescence, dropping a hint that RL got something to do with absorption spectra and was a novel luminescence technique. In addition, RL, to a certain degree, was also disclosed to explore structural characteristics. For example, the maximum RL peaks of rhodamine B (RhB) and rhodamine 6G (Rh6G), were at 571 and 540 nm, respectively, presumed probably due to different substituents contained on the conjugated systems of Rh6G and RhB.

It is a pity that Liu et al. at that time did not elucidate the essence of RL technique, did not compare it with RLS technique [3–6] because these two techniques shared the same performance, and their work has been absorbing few attentions from other analysts even until now.

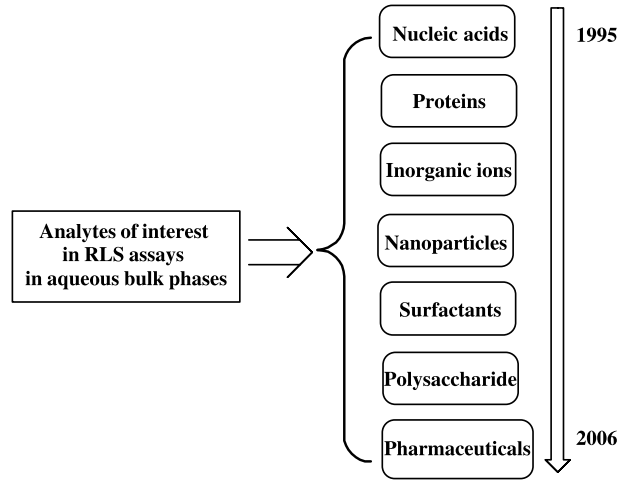

Fig. 3. Analytes of interest and their rough time ranking in RLS homogenous assays

The contribution from Li et al.

In 1996 and 1997, Li et al. first applied RLS technique in biochemical assays [18, 19]. It was disclosed that the obviously enhanced scattering peaks were both set to the corresponding absorbance envelopes of self-assembled chromophores on nucleic acids and that with the enhanced RLS signals nucleic acids could be determined satisfactorily. However, we think it more important and interesting that it was first found besides molecules with large rings, such as porphyrins [3, 4, 6] and chlorophyll a [5, 6], some other molecules, such as 4-[(5-chloro-2-pyridyl)azo]-1,3-diaminobenzene (5-Cl-PADAB) [19], could be taken as RLS probes as well. Rapidly, these two investigations [18, 19] aroused much interests from lots of other analysts, greatly prompting RLS applications in analytical chemistry.

Nevertheless, it is pitiful that Li et al. then did not talk about RLS heterogeneous assays, did not contrast it with RL technique [13] and did not advance its theory, either.

Since then, RLS technique, exactly traditional RLS technique termed by us and talked about below, has been being dominantly and more and more applied in homogenous assays of considerable analytes, chiefly including, as Fig. 3 shows, proteins [9, 20–25], nucleic acids [11, 18, 19, 26, 27], polysaccharide [10, 17, 28] pharmaceuticals [29, 30], nanoparticles [2, 31, 32], ions [14–16, 33, 34] and surfactants [35, 36]. In addition, Fig. 3 also demonstrates the approximate ranking of analytes interested in RLS homogenous assays.

Its basic theory (1998–2000)

As Fig. 2 shows, from 1998 to 2000, discussing RLS theory is a characteristic work. Before coming into this topic, we consider it necessary to first clarify the spectral criterion of judging RLS because some analysts until now still believe that light scattering experiments, all performed on a CFS by simultaneously scanning both monochromators, are all ascribed to RLS.

As Pasternack et al. specified, RLS was referred to an enhancement of scattered light in close proximity to an absorption band [3–6]. Consequently, judging whether a light scattering is ascribed to RLS or not is merely based on whether an enhanced scattering peak is near or within some absorption band of a scattering system or not. Furthermore, for some scattering systems, it has proved that conventional Rayleigh scattering [37, 38] and large particle light scattering [39, 40], in both of which enhanced scattering peaks are independent of absorption bands at all, could be acquired according to the same performance as that of RLS. And in 2000, based on many practices Li et al. [41] definitely judged it surely improper that considering all light-scattering, performed on a CFS with the same procedure as that of RLS , as RLS ones. As a result, we consider it somewhat inexact that in a review [42], as described in the first sentence of the abstract, by coupling and scanning simultaneously excitation and the emission monochromators of a common spectrofluorometer, enhanced RLS signals could be obtained.

Figure 4 is proposed to illustrate the spectral feature of RLS. Figure 4 (left) forms a striking contrast to Fig. 4 (right) in the relationships between the scatter-

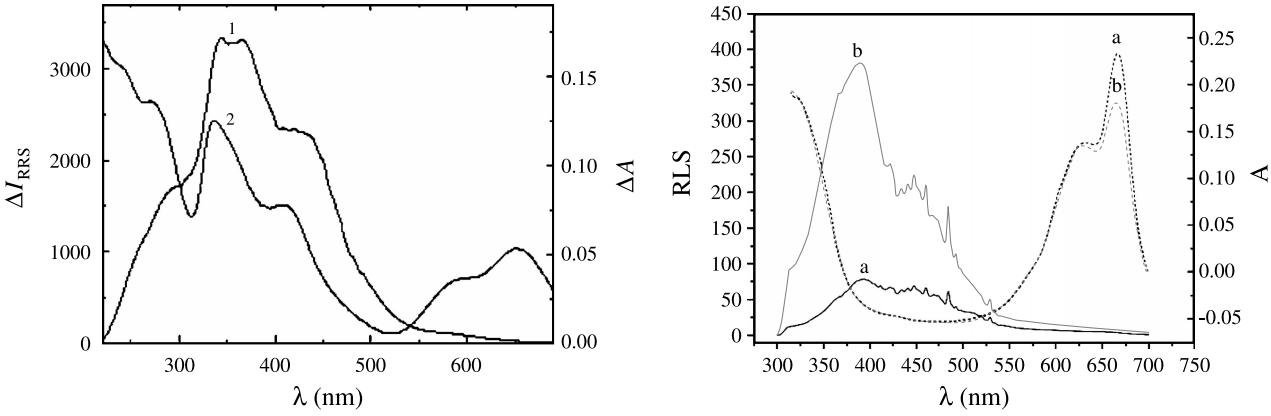

Fig. 4. Representative spectral relationship between a RLS/RRS profile and an absorption profile (left) in contrast to that between a non-RLS/RRS profile and an absorption profile (right). *Left*: Comparison of spectra characteristics of RRS and absorption for DOTC-UO_2^{2+}-WB. (*1*) Absorption spectrum; (*2*) RRS spectrum. Curves 1 and 2 are compared with reagent blank (UO_2^{2+}-WB). The concentrations of DOTC, UO_2^{2+}, and WB are 1.0×10^{-5}, 5.0×10^{-6}, and 1.5×10^{-5} mol L^{-1} respectively. Reprinted from [29] with the permission from Elsevier Inc. Copyright 2005, Elsevier Inc. *Right*: RLS (—) and absorption (. . .) spectra of CoTSPc – BSA. CoTSPc: 1.0×10^{-5} mol L^{-1}, pH 4.0. BSA: (*a*) 0.0 μg mL^{-1}; (*b*) 5.0 μg mL^{-1}. Reprinted from [38] with the permission from Elsevier Inc. Copyright 2004, Elsevier Inc.

ing and the corresponding absorption spectra of two different interacting systems. For the ternary complex of doxytetracycline (DOTC)-uranyl acetate (UO_2^{2+})-water blue (WB), shown in Fig. 4 (left), the maximum scattering peak (343 nm) is located closely to the maximum absorption peak (about 340 nm), obviously ascribing to RLS. However, for the binary complex of cobalt-tetrasulfonatophthalocyanine (CoTSPc) and bovine serum albumin (BSA), displayed in Fig. 4 (right), the enhanced scattering occurs where the absorption decreases, definitely not ascribed to RLS. In order to further specify the spectral differences between RLS and non RLS based on a CFS, Table 1 is offered to show more other examples. Furthermore, typical RLS effects of some kinds of analytes, such as polysaccharide [28], pharmaceuticals [30] and ions [16] have been also supplied to strengthen the essential spectral feature of RLS, displayed in Fig. 5.

As above described, RLS is actually a RRS in most situations. Moreover, it is worth noting that Pasternack et al. [3] also implied that the proposed RLS was a special Rayleigh scattering. As a result, the basic theory of RRS should be discussed here. At present, three representative explanations to RRS technique have been made:

(I) The one, came up with by Pasternack et al. in 1993 [3]. Although this explanation to RLS was not made during the period from 1998 to 2000, it is the mainstream in explaining RLS. So, the explanation from Pasternak et al. should be discussed.

Table 1. The disclosure of the spectral discriminations among resonance light scattering (RLS), conventional Rayleigh scattering and large particle light scattering with the use of some assay practices of nucleic acids and proteins

Names of light scattering	Light-scattering systems			
	Description	λ_{abs} (nm)	λ_{sca}^{max} (nm)	Ref.
RLS	(1) The interactions of nucleic acids with TAPP	412	near 432	[18]
	(2) The interaction of DNA with TAPP and BPR	412	434	[26]
	(3) The interactions of nucleic acids with 5-Cl-PADAB	545	547	[19]
Conventional Rayleigh scattering	(1) The interactions of proteins with ACBK	near 500	345	[37]
	(2) The interactions of proteins with Ag25	over 600	347	[38]
Large particle light scattering	The interactions of nucleic acids with protamine sulfate	no obvious absorption near 365	365	[39]

All these measurements were performed on a conventional fluorescence spectrophotometer with the same procedure described in the text
λ_{abs}: wavelength of some absorption band; λ_{sca}^{max}: wavelength of maximum scattering peak; *BPR* bromopyrogallol red; *VB4R* victoria blue 4R; *ACBK* acid chrome blue K; Ag25 acid green 25.

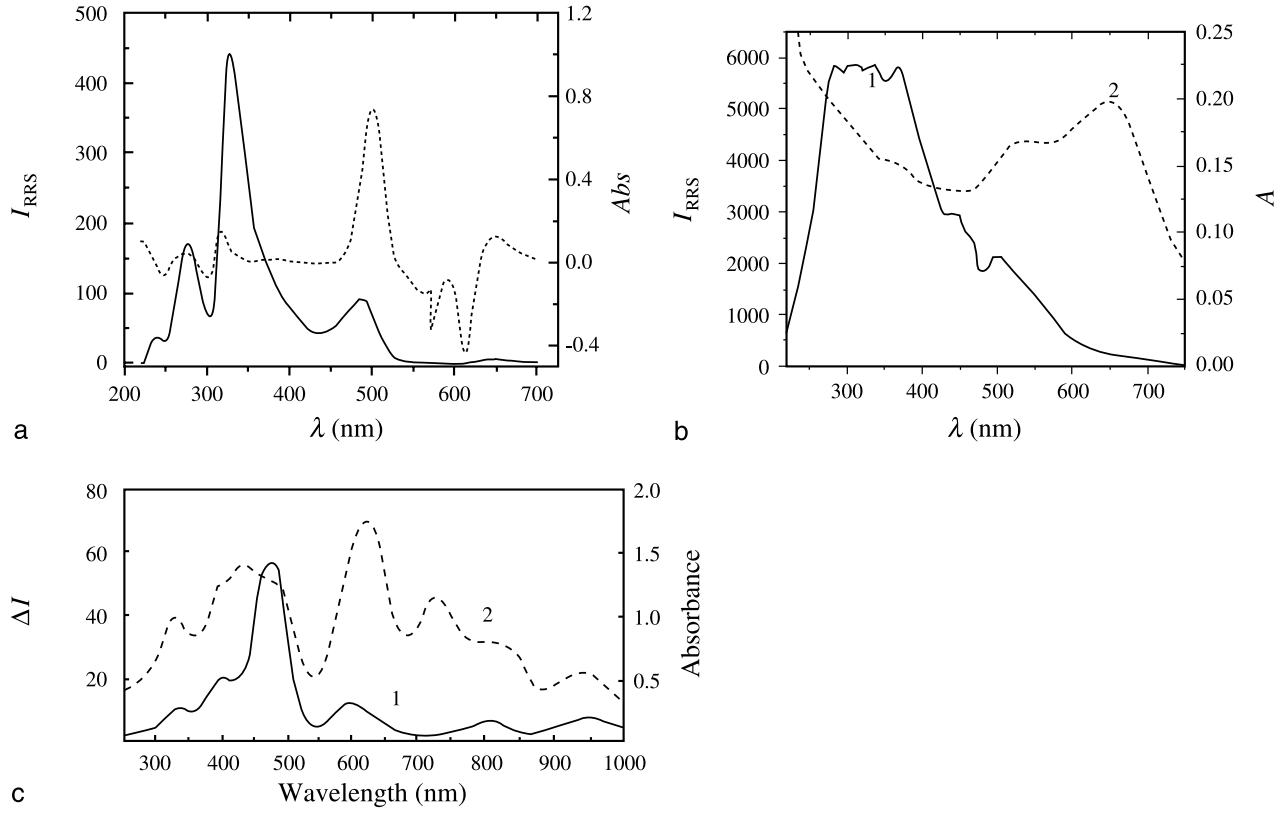

Fig. 5. Typical RLS effects of some types of analytes. (a) RRS spectrum (—) and absorption spectrum(. . .) of the CSA-CV system. *CSA* Chondroitin sulfate A; *CV* crystal violet. Reprinted from [28] with the permission from WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. Copyright 2005, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. (b) Comparison of the spectral characteristic for RRS and absorption (*1*) RRS spectrum; (*2*) absorption spectrum against the water blank [Au] = 4.85×10^{-5} mol L^{-1}, [VB1] = 2×10^{-7} mol L^{-1}, pH 4.0. VB1: vitamin B1. Reprinted from [30] with the permission from Springer Science and Business Media. Copyright 2006, Springer Science and Business Media. (c) RRS and absorption spectra of [MG] [I_3^-]: (*1*) RRS spectrum; (*2*) absorption spectrum. MG: malachite green. Reprinted from [16] with the permission from Elsevier Science B.V. Copyright 1999, Elsevier Science B.V.

(II) The one, first put forward by Bauer et al. [43], Miller [44], Anglister and Steinberg [45], and then representatively expounded by Liu et al. [9, 14, 15, 17] and Li et al. [12, 37, 40] after 1997.

(III) The one, suggested by Jiang in 2003 [46].

The idea from Pasternack et al.

According to us, the idea from Pasternack et al. [3–6] can be summarized as two basic points:

(I) Complex refractive index, m, is fairly employed to study light scattering near absorption bands as m had been introduced in study of Rayleigh scattering near absorption bands [43, 44] in 1970s. And for a Rayleigh scattering system, its absorption cross section (C_{abs}) and scattering cross section (C_{sca}), can be simply calculated as

$$C_{abs} = (\pi\gamma^2)4\chi Im[(m^2-1)/(m^2+2)] \quad (1)$$

and

$$C_{sca} = (\pi\gamma^2)(8/3)\chi^4[(m^2-1)/(m^2+2)]^2 \quad (2)$$

in which γ is the scatterer radius, *Im* is the imaginary part within the parenthesis, misunderstood at one time [18], and χ is the size parameter, equal to $(2\pi\gamma n_{med})/\lambda_0$, where λ_0 is the incident wavelength and n_{med} is the refractive index of the medium. Although when λ_0 approaches absorption bands, m increases anomalously, C_{sca} is a much stronger function of m than C_{abs} according to Eqs. (1) and (2). As a result, enhanced light scattering takes place in that case. However, enhanced light-scattering signals, developing from only the increase of m when λ_0 is moving toward absorption bands, is not enough to be detected with a CFS. It is wise of Pasternack et al. to find a way to solve it.

(II) Self-aggregations or self-assemblies of chromophores are formed in investigated systems, which,

as an important innovation and a vital light-scattering enhancement factor, are distinguished from RRS researches before 1993, using diphenylpolyenes [43], lycopene [45], β-carotene [47], dibenzoylmethane and its selected diorganotin(IV) complexes [48], cyanine dyes [49], nitrophenolate anions [50] and heme proteins [51] as examples, of which light-scattering signals are too weak to be measured with a CFS. The contribution of self-aggregations or self-assemblies of chromophores to the great light-scattering enhancement results from the fact that in a systems with a fixed concentration, the volume of the scatterer (v), influences the amounts of absorption, $A(\lambda_0)$, and scattering, $I_{sca}(\lambda_0)$, in very different ways. Since $v = (4/3)\pi\gamma^3$ for a spherical scatterer, $I_{sca}(\lambda_0)$ and $A(\lambda_0)$ of a sample of thickness L can be calculated with the following expressions (For $I_{sca}(\lambda_0)$, often used orthogonal detection geometry is considered), respectively,

$$\begin{aligned} I_{sca}(\lambda_0) &= C_{sca}\left(\frac{N}{V}\right)I_0(\lambda_0) \\ &= \frac{24\pi^3 v^2 n_{med}^4}{{\lambda_0}^4}\left(\frac{m^2-1}{m^2+2}\right)^2\left(\frac{N}{V}\right)I_0(\lambda_0) \\ &= \frac{24\pi^3 v^2 n_{med}^4}{{\lambda_0}^4}\left(\frac{m^2-1}{m^2+2}\right)^2\left(\frac{\frac{m'}{\rho v}}{V}\right)I_0(\lambda_0) \\ &= \frac{24\pi^3 v n_{med}^4}{{\lambda_0}^4}\left(\frac{m^2-1}{m^2+2}\right)^2\left(\frac{m'}{\rho V}\right)I_0(\lambda_0) \\ &= \frac{24\pi^3 v n_{med}^4 I_0(\lambda_0)}{\rho{\lambda_0}^4}\left(\frac{m^2-1}{m^2+2}\right)^2\left(\frac{n'M}{V}\right) \\ &= \frac{24\pi^3 v n_{med}^4 M I_0(\lambda_0)}{\rho{\lambda_0}^4}\left(\frac{m^2-1}{m^2+2}\right)^2 = Kc \end{aligned} \tag{3}$$

$$\begin{aligned} A(\lambda_0) &= 2.303^{-1}\left(\frac{N}{V}\right)C_{abs}L \\ &= 2.303^{-1}\left(\frac{N}{V}\right)\frac{8\pi^2\gamma^3 n_{med}}{\lambda_0}\mathrm{Im}\left(\frac{m^2-1}{m^2+2}\right) \\ &= 2.303^{-1}\left(\frac{N}{V}\right)\frac{6\pi v n_{med}}{\lambda_0}\mathrm{Im}\left(\frac{m^2-1}{m^2+2}\right) \\ &= 2.303^{-1}\left(\frac{\frac{m'}{\rho v}}{V}\right)\frac{6\pi v n_{med}}{\lambda_0}\mathrm{Im}\left(\frac{m^2-1}{m^2+2}\right) \\ &= 2.303^{-1}\left(\frac{m'}{\rho V}\right)\frac{6\pi n_{med}}{\lambda_0}\mathrm{Im}\left(\frac{m^2-1}{m^2+2}\right) \end{aligned} \tag{4}$$

where N/V is the number of scatterers per unit volume, m' is the mass of the scattering material, ρ is the density of scattering material and $I_0(\lambda_0)$ is the incident intensity, and n', M and c are amount of substance, molar mass and molar concentration of the scattering material, respectively. As Eq. (3) displays, under certain conditions, $I_{sca}(\lambda_0)$ is directly proportional to v, which, as proved late by Yguerabide et al. [2], will give rise to the obvious enhancement of C_{sca}, but $A(\lambda_0)$ is independent of v. Here, we should clarify that some researchers [12] believe that light-scattering signals under certain conditions are in direct proportion to v^2, which may be a misunderstanding to RLS theory proposed by Pasternack et al. [3–6]. Besides, Eq. (3) also displays the quantitative basis of RLS technique and RLS properties even of a same scattering system can be dissimilar more or less based on various CFSs because $I_0(\lambda_0)$ has a strong relation with instrumental conditions.

Now, it is effortless for us to understand the mechanisms of conventional Rayleigh scattering [37, 38] and large particle light scatterings [39, 40], with Eq. (3), which is due to merely much increased v in developing scatterers and why a laser source have to been utilized in RRS detections before 1993, which, with Eq. (3), expects to enlarge light-scattering signals as much as possible. We figure that the idea from Pasternack et al. on RLS theory is efficient, but it is a pity for them not to specify the contributions of real and imaginary parts of m to the enhancement of RLS, respectively.

The idea from Liu et al. and Li et al.

In the opinion of us, the characteristic of Liu et al. [9, 14, 15, 17] and Li et al. [12, 40, 41] discussing on RLS theory is to combine m with Kronig–Kramers equation to elucidate the functions of real (n) and imaginary (k) parts of m in RLS of a scattering system with c, respectively, which is a good complement to the idea from Pasternack et al. [3–6] and is thus talked over here.

Since the expression of m can be given as below [44]

$$m = n - ik \tag{5}$$

in which n and k portray effects of refractive and absorption of a scattering system, respectively, and $i = \sqrt{-1}$, the relationship between n and molar absorption coefficient ($\varepsilon(\lambda)$) at any wavelength within the absorption band can be described with Kronig–Kramers equation [47]:

$$n = n_{med} + \frac{2.303c{\lambda_0}^2}{2\pi^2}\int_0^\infty \frac{\varepsilon(\lambda)}{{\lambda_0}^2 - \lambda^2}d\lambda \tag{6}$$

Therefore, the increment of n with the fluctuation of c, is given as

$$\frac{\partial n}{\partial c} = \frac{2.303\lambda_0{}^2}{2\pi^2}\int_0^\infty \frac{\varepsilon(\lambda)}{\lambda_0{}^2 - \lambda^2}d\lambda \tag{7}$$

As for k, it can be expressed as the following equation [47]:

$$k = \frac{2.303\varepsilon(\lambda_0)c\lambda_0}{4\pi} \tag{8}$$

in which $\varepsilon(\lambda_0)$ was the molar absorption coefficient at λ_0. So, the increment of k with the fluctuation of c can be specified by

$$\frac{\partial k}{\partial c} = \frac{2.303\varepsilon(\lambda_0)\lambda_0}{4\pi} \tag{9}$$

According to Eqs. (7) and (9), with λ_0 withdrew from absorption bands, $\partial n/\partial c$ and $\partial k/\partial c$ both appear weaker. Nevertheless, with λ_0 moving towards absorption bands, they both get increased. Especially, it is worth noticing how they behave in high and low frequency sections of absorption bands, respectively. Specifically, in high frequency section $\partial n/\partial c$ counteracts $\partial k/\partial c$ because $\partial n/\partial c$ is negative but $\partial k/\partial c$ is positive, whereas in low frequency section $\partial n/\partial c$ accords with $\partial k/\partial c$ because both $\partial n/\partial c$ and $\partial k/\partial c$ are positives. Especially, when λ_0 is slightly more than λ_{max}, m reaches maximum, explaining why RLS is strictly termed pre-resonance light scattering [52].

Besides, Liu et al. also believe that since RLS is ascribed to a synchronous luminescence [13], i.e. $\lambda_{ex} = \lambda_{em}$, $I_{sca}(\lambda_0)$ should follow the equation [53, 54]:

$$\begin{aligned} I_{sca}(\lambda_0) &= k'cbE_{ex}(\lambda_{ex})E_{em}(\lambda_{ex}) \\ &= k'cbE_{ex}(\lambda_{em})E_{em}(\lambda_{em}) \end{aligned} \tag{10}$$

where λ_{ex} and λ_{em} are the excitation and emission wavelengths, respectively, k' is a constant closely associated with instrumental conditions, b is the pathlength, $E_{ex}(\lambda_{ex})$ and $E_{em}(\lambda_{ex})$ are functions of the incident and emitted beams, respectively. Obviously, Eq. (10) shows that when other variables keep constant, $I_{sca}(\lambda_0)$ is directly proportional to c, and also supports that RLS signals are closely associated with instrumental conditions. Nonetheless, the prominent disadvantage of Eq. (10) is that it is difficult to elucidate the causes of enhanced RLS.

The idea from Jiang

Jiang strongly concentrated on studying various nanoparticles [32, 46, 55, 56] with RLS. His explanation to RLS is based on two aspects: (I) a new classification of scatterers and (II) the introduction of de Broglie wave.

(I) According to Jiang [46, 57], since RLS is strongly related to light absorption, scatterers should be divided into three types:

(a) White scatterers, characterized by scattering all of exciting light of any wavelength always with the same efficiency. White scatterers do not really exist, but scatterers can be regarded as quasi white ones when λ_0 is far away from their absorption bands. For instance, human serum albumin (HSA) and calf thymus DNA (ctDNA) can be treated as white scatterers well from 200 to 1000 nm. Importantly, light-scattering properties of white scatterers, reflecting well intensity profiles of exciting sources, can not disclose their chemical components. Surely, not RLS but conventional Rayleigh scattering or large particle light scattering can be properly used to handle systems of white scatterers, which cannot be proved with frequently used Spectrophotometry and Molecular fluorescence.

(b) Black scatterers, absorbing all exciting light of any wavelength and emitting no scattered light. Surely, black scatterers do not exist either, but some nanoparticles, for instance, prepared Chinese ink and black Pt nanoparticles of relatively high concentrations, can be considered as black scatterers.

(c) Gray scatterers, both absorbing and scattering light, which can not scatter exciting light evenly. Their light-scattering properties are remarkably influenced by their chemical components because different chemical components own diverse absorption spectra, affecting the corresponding scattering spectra. Obviously, gray scatterers are most interesting in RLS studies. However, it has proved that not all gray scatterers can produce RLS [37, 38]. So, we believe that it is very interesting and necessary to subdivide gray scatterers to locate scatterers with some same characteristics, definitely giving rise to RLS.

(II) As presumed [57], whether nanoparticles or supramolecules act as scatterers, they can both produce de Broglie wave, characterized by the following equation,

$$P = m''u \tag{11}$$

in which m'' is the mass of an electron on the surface of a NP or supramolecule, u and P are the speed and momentum of this electron, respectively. Therefore, the wavelength of de Broglie wave (λ_d) emitted by a NP or supramolecule, can be expressed by

$$\lambda_d = \frac{h}{P} = \frac{h}{m''u} \quad (12)$$

For different nanoparticles or supramolecules, they own special λ_d, and when λ_0 get resonant with λ_d, RLS takes place.

According to us, this point of view is fresh and possible to pioneer another approach to explore RLS theory, but at present it is surely very infant because it cannot provide satisfactory explanations to many problems, such as the causes resulting in conventional Rayleigh scattering [37, 38] and large particle light scattering [39, 40], and the quantitative base of RLS technique.

Resonance light-scattering (RLS) probes

The discovery and selection of RLS probes are of much significance because it is important to acquire satisfactory RLS signals. As displayed in Fig. 2, the development of RLS Particles^{TM} is concentrated upon between 1998 and 2000. Before talking about RLS Particles^{TM}, it is necessary to conclude the basic traits of RLS labels because it will make us know well why RLS Particles^{TM} is superior to other RLS labels.

Its fundamental features

We have been noticing that Pasternack et al. and many other researchers nearly concentrate on either porphyrins [3, 4, 6, 58–68] or chlorophyll [5, 69–71] in their RLS studies, all distinguished by chromophores with large molar absorption coefficients and formations of extended electronic-coupling self-aggregations or self-assemblies in the presence of polymeric matrices, such as nucleic acids [3, 6, 58, 59, 61, 62, 64, 65] and proteins [59, 60]. Moreover, it has been confirmed that not all small organic molecules (SOMs), considered by us to be mostly used in assays with light-scattering techniques with a CFS at present, can be used as RLS probes. These all imply that RLS probes should meet some characteristics distinguished from non-RLS ones. According to reported RLS probes, especially by Pasternack et al. [3–6], Liu et al. [10, 15–17] and Li et al. [18, 19], we conclude that they are supposed to have the following basic characteristics:

(I) In possession of chromophores with proper molar absorption coefficients, which are also required to build up ''good'' electron couplings, first considered by Pasternack et al. [3–6], and to increase structural rigidity and coplanarity in the case of self-assembling on targeted analytes, predicted with the AM1 method of quantitative chemistry and proved by experimental results [10], therefore making probability of transition of electron increased but probability of molecular relaxation diminished. In such scattering systems, m will increase sharply when the incident beam is approaching absorption bands, causing that C_{sca} gets its maximum where C_{abs} behaves just the same as well, in essence giving rise to RLS. This term assures an essential discrimination between RLS and non-RLS techniques, for instance conventional Rayleigh scattering [37, 38] and large particle light scattering [39, 40], of which locations of enhanced scattered peaks are foreign to those of absorption envelopes.

(II) In possession of large sufficient size, accordingly developing scatterers with obvious enlargements in volume in the presence of analytes, which is certified as a considerable magnification effect upon RLS signals [3–6] and is distinguished form RRS prior to 1993. This term ensures that RLS can be simply exercised on a CFS.

(III) In possession of compatibilities in structural characteristics and physical properties, such as symmetry, charges and solubility, with those of targeted analytes in appropriate medium conditions, which regularly takes acidity and ionic strength into account. This term makes it sure that probes can combine with targeted analytes firmly and therefore can radiate stable scattered light because without it volumes of scatterers cannot keep relatively stable, making scattering signals disagreeably fluctuant.

Above all, it is worthy of being noticed that a probe, merely satisfying (II) and (III), can be appropriately employed for non-RLS techniques, such as conventional Rayleigh scattering and large particle scattering, and a probe, just meeting (I) and (III), can be fairly used as a RLS one, but it works satisfactorily necessarily with the aid of a laser source.

The development of resonance light-scattering (RLS) particles^{TM} (1998–2000)

To date, RLS probes for assays come by and large from SOMs and at times from metal nanoparticles, for instance rhodamine dyes [13, 72], porphyrins

[18, 25, 52], 5-Cl-PADAB [19], basic triphenylmethane dyes [15], basic phenothiazine dyes [73], basic diphenyl naphthylmethane dyes [9], colloidal metal particles [23, 24, 27, 55, 56, 74–76] and cationic acridine dyes [77], of which, however, RLS Particles™ has been found most effective.

Its brief description. In 1998, submicroscopic particles, such as nano-scaled gold and silver particles, were discovered to generate scattered light stably, clearly and efficiently, which, accordingly, were believed to be used as novel and ultrasensitive tracers in clinical and biological applications [2, 31]. Soon, spherical nano-sized colloidal metal particles of uniform dimension (generally gold or silver particles with their diameters between 40 and 120 nm), playing an important part in a U.S. patent (U.S. Patent 6,214,560) named *analyte assay using particulate labels* [78], were commercially described as RLS Particles™.

Its advantages. Compared with SOMs, characterized by molecular chromophores, and chiefly according to the corresponding investigations of Yguerabide et al. [2, 31, 79], the main advantages of RLS Particles™ are talked over by us as the following:

(I) The ability to scatter light most efficiently. Gold nanoparticles, with particle concentrations as low as 10^{-16} M in a suspension or as low as 0.005 particles μ^{-2} on a transparent surface (both depending on particle size), with a simple illuminator, their scattered light can be detected by unaided eye for qualitative measurements or by a simple light-sensitive detector for quantitative measurements [2, 79]. Thus, this advantage is considered by us to support both ultra-sensitivity and less use of labels. A case in point [27] is that in the detection of DNA hybridization with 80-nm-diameter gold nanoparticles coated with anti-biotin antibodies, it was found that with 1 ng of probe (16.7 pg μL^{-1}), no detectable signal was observed in the Cy3 image, whereas significant signal was without doubt detected in RLS image. Equally important, also thanks to this merit, it is practical to make use of relatively simple instrumentation in RLS assays. One of representative case is that individual RLS Particles™ can be observed in an inexpensive student microscope with a 100 W filament lamp, allowing for sensitive and convenient particle counting assays [79]. However, in the RLSI detection of proteins [80] with α, β, γ, δ-tetrakis(p-sulfophenyl)porphyrin ($TPPS_4$), not only an Olympus IX70 inverted microscope but also an Argon ion laser source are necessary.

(II) The ability to prepare a range of functional probes. In contrast to SOMs, RLS Particles™ can be amenably conjugated (coated) with diverse substances though thiol and amino chemistry [74, 75, 79], such as antibodies, protein receptors, DNA probes, ligands and more, attaching to specific analytes, to make functionalized ones, avoiding non-specific binding in clinical and biological assays. As an example [79], 80 nm gold antibiotin IgG conjugate particle can effectively label breast carcinoma tissue, which is chiefly on the surface membranes of the cells where the tyrosine kinase of HER-2/neu, a proto-oncogene and the human analog of the rat neu gene associated with rat neuroblastoma, is located. We think that this feature can advance immuno-RLS assays, which is an interesting field but is not studied and applied widely at present.

(III) The high stability of signals. In contrast with RLS in the presence of SOMs and fluorescence [27, 81], with repeated or continuous exposure to intense light sources, RLS signals in the presence of RLS Particles™ are not prone to photobleaching, quenching or decaying, thus allowing experimental results to be stably and permanently archived. Therefore, a high reproducibility for both intra and inter assays [82] can be obtained.

(IV) The easy variation of colors of scattered light. Unlike SOMs, with configured white light, colors of scattered light of RLS Particles™ can be conveniently changed by varying their particle sizes [2, 31], compositions [83], morphologies [83] or structures [83]. For instance, gold nanoparticles, displaying various colors, such as green, yellow, and orange, can be readily prepared by merely changing their diameter from 40 to 120 nm [2, 31]. Based on this advantage, it was anticipated by Lahiri et al. [27] that RLS Particles™ could be acted as multicolor labels in a wide variety of assays [27], which, relative to single-color RLS and fluorescence detection, will enable more advantages, such as the ability of measuring smaller changes in differential gene expression analyses. Fortunately, in 2003, Two-Color Nucleic Acid Microarray Toolkit™

[84] was developed, realizing labeling of two different samples with separate labels and simultaneous hybridization of both of them to the same microarray. As it was proved [84], Two-Color Nucleic Acid Microarray Toolkit™ can accurately determine differential gene expression using a 2-fold cut off at total RNA input levels, where positive signals are undetectable by fluorescence, and using a 2-fold difference in expression as the threshold for measuring differential gene expression, three times as many genes can be determined to be differentially expressed using two-color RLS detection than with fluorescent detection, confirming the anticipation of Lahiri et al. [27].

(V) The long conservation life. Vis-à-vis SOMs, when properly prepared, whether bare (uncoated) RLS Particles™ or coated ones both have a long shelf life without aggregation or deterioration [79, 85].

In summary, according to us, RLS Particles™, with these advantages, is superior to SOMs and is poised to open out great potentials in RLS applications.

Its light-scattering essence. Unlike SOMs, RLS Particles™ in fact belongs to plasmon resonant particles (colloidal gold or silver nanoparticles with typically 10–120 nm in diameter) [86, 87], and their brilliant colors result from their strong UV-Visible absorption bands (plasmon absorption bands), which, however, are not present in bulk metals [85]. Namely, when the frequency of an incident light is in resonance with that of the collective excitation of conductive electrons (plasmons) of RLS Particles™, a strong UV-Visible absorption band results and at the same time the strong energy absorbed is radiated in the form of an electromagnetic wave with the same frequency as that of the incident light, termed ''localized surface plasmon resonance'' (LSPR).

Now, in combination with the basic traits of RLS robes above described, it is without difficulty to comprehend why RLS Particles™ is much better than SOMs in RLS assays. Exactly, besides the factors that the volume of a single RLS Particles™ is much larger than that of an SOM and that RLS Particles™ is easily promoted to functional ones, the presence of strong UV-Visible absorption bands in RLS Particles™ results in extreme molar extinction coefficients (ca. $3 \times 10^{11}\,M^{-1}\,cm^{-1}$) [85], far exceeding those of SOMs and influencing light-scattering intensities more significantly near strong UV-Visible absorption bands.

Derived techniques from resonance light-scattering (RLS) (2001–2004)

As Fig. 2 showed, the presence of a variety of RLS derived techniques characterizes the advance of traditional RLS technique between 2001 and 2004, which is referred by us to be applied in the models of homogenous liquid phase assays, representatively offered by Li et al. [18, 19], and heterogeneous assays, characteristically proposed by Yguerabide et al. [2, 31, 79].

Prior to discussing RLS derived techniques, it is very necessary to make a brief overview of traditional RLS applications from 1995 to the present, because it implies the impetuses and intensions of suggestions of RLS derived techniques. In addition, we should clarify that the advantageous considerations of RLS derived techniques in contrast to traditional RLS technique are all proper to be exercised to improve non-RLS techniques.

An overview of traditional resonance light-scattering (RLS) technique

After studying the corresponding published papers, we have found the following fundamental points.

(I) In contrast with breakthrough papers, as considered by us and displayed in Table 2, other papers appear regular to some extent, but they are still of much value and interest, such as in studying interactions of biopolymers or pharmaceuticals with SOMs, which is a useful way to investigate possible targeted mechanisms between drugs and biopolymers and to screen pharmaceuticals, hunting for tags for same targets and draw a comparison between them, which is advantageous in summarizing favored structures of tracers and further synthesizing more powerful ones, expanding ranges of analytes of being focused on, which is positive to bring traditional RLS technique into more fascinating scopes, and uncovering the limitations of RLS technique, which is beneficial to impel the progress of traditional RLS technique.

(II) Most determinations are executed in the model of homogenous assay by binding analytes generally with SOMs [8, 9, 11, 18, 19, 20–22, 25, 26, 28–30, 33–36] or sometimes with metal nanoparticles [2, 23, 31]. At present, only several papers are beyond the confine. Here, we should mention that it is Yguerabide et al. [2, 31, 79] who succeeded to not only pull out RLS appli-

Table 2. The breakthrough papers on light-scattering techniques based on conventional fluorescence spectrophotometers (CFSs)

No.	Authors	Time	Breakthrough	Ref.
1	Liu et al.	1995	Discovering the phenomena of resonant luminescence in ion-association of rhodamine dyes with some metal ions	[13]
2	Li et al.	1996	Using RLS technique for biochemical assays, and therefore published as a technical note	[18]
3	Li et al.	1997	Confirming the fact that some other molecules could also serve as RLS labels besides porphyrins and chlorophyll a	[19]
4	Li et al.	1997	Signals of conventional Rayleigh scattering instead of RLS could be obtained with the same procedure with that of RLS	[37]
5	Yguerabide et al.	1998	1. Proposing RLS Particles™ 2. Establishing formulae of describing the scattering properties of RLS Particles™ with simulate use of those of handling fluorescence 3. Detailing the differences between RLS and fluorescence 4. Commence RLS applications in cell biology 5. Launching on heterogeneous assays of RLS 6. Setting about imaging analysis of RLS	[2] [31]
6	Huang et al.	1999	Proposing the three-dimensional RLS spectra, simultaneously displaying signals of Raman scattering, second-order scattering and frequency doubling scattering besides RLS ones	[11]
7	Li et al.	1999	Implementing large particle light-scattering technique	[39]
8	Liu et al.	1999	Employing chemometrics to understand charge distribution of RLS labels before and after their interacting with analytes to explore the essence of enhanced resonance Rayleigh scattering	[15]
9	Yguerabide et al.	2001	Enriching the contents of RLS assays in cell biology and pioneering the contents of RLS assays in histology	[79]
10	Lahiri et al.	2002	Combing microarray technique with RLS technique for assays	[27]
11	Liu et al.	2002	Describing frequency doubling scattering and second-order scattering in an international journal	[17]
12	Brust et al.	2005	Assaying protein functionality (kinase activity), and therefore published as a technical note	[24]

cations from simplex liquid phase homogenous assays but also find more targets of much interest, such as cell surface components [79], and more application formats, such as micoarrays, microfluidics and solid phase, in which RLS Particles™ is much favored. Besides, Lahiri et al. [27] and Wang et al. [24], respectively, detected DNA hybridization and proteins as well as enzyme functionality in microarrays with functional RLS Particles™.

(III) Most assays are carried out with permanent right-angle geometry satisfactorily. However, RLS of non-90° has not been attempted yet, making it impossible to compare RLS of 90° with that of non-90° and further to disclose the analytical potentials of RLS of non-90°.

(IV) Most determinations do not involve correction practices. However, as declared by McPhie [88], RLS profiles must be corrected for instrument performance, because the RLS was observably influenced (denatured) by instrumental properties, such as the variational intensities of lamps at any wavelength, easily causing that RLS profiles showed similarity to those of the lamps and even that enhanced scattering peaks could be detected only as a result of enhanced incident intensities.

(V) Most RLS applications are absorbed in the changes of scattering signals before and after the combinations of analytes with corresponding RLS markers, but leave out studies of imaging thus-formed RLS scatterers, considered interesting in exploring RLS potential in disease diagnosis [31, 42, 79].

(VI) RLS homogenous assays are particularly time-consuming as a result of being lack of automation in the input of samples and the compulsive detection of samples after establishing interacting equilibriums between analytes and corresponding RLS tags, which is not suitable for the assays of large numbers of samples and on-line

assays. Besides, it also suffers from expending too much samples for completing a project, which is not fit for rare samples.

(VII) RLS detections have scarcely been applied to characterize various kinetic processes.

Total internal reflection resonance light-scattering (TIR-RLS) technique

In 2001, Huang et al. proposed a TIR-RLS technique to probe amphiphilic species at an aqueous/oil interface [89] in situ, with which proteins [90], nucleic acids [91–93] and pharmaceuticals, such as thiamine [94], berberine [95] and penicillin [96, 97] at aqueous/oil interfaces were analyzed satisfactorily.

Its basic theory. We summarize the basic TIR-RLS theory as the following points. Specifically,

(I) Constructions of amphiphilic species. There are three ways to create amphiphilic species, absorbed well at the aqueous/oil interface [98, 99], in an aqueous/oil system. The first way is through an interfacial interaction with two species of water-solubility and oil-solubility, respectively. It was uncovered that in an aqueous phase chlortetracycline (CTC) could interact with Eu(III), which could combine with trioctyl phosphine oxide (TOPO) in an oil phase, to develop an ternary amphiphilic species [89]. The second way is by way of co-adsorption with surfactants, presumed to reside well at aqueous/oil interfaces [100, 101]. As reported [94], in an aqueous medium thiamine could bind with dodecylbenzene sulfonate (SDBS), sodium dodecylsulfonate (SDS) and sodium lauryl sulfate (SLS), respectively, to make a newly developed amphiphilic species. The third way is by means of synergistic adsorption. As revealed [97], penicillin and berberine, both owning amphiphilic structures, could form a newly built amphiphilic species after their interaction.

(II) An evanescent wave (field) as exciting source, developed at the interface when an beam directly from a CFS is incident from an aqueous phase to an oil phase and is totally reflected [102]. Since the intensity of the evanescent wave decays exponentially with increasing distance from the interface, falling to undetectable levels within less than one wavelength, the chemical or biological species in the interfacial region can be highly selectively excited [103].

(III) The quantificational basis of TIR-RLS. It is worth mentioning that Feng [104], with the expression of scattering intensity on an optical surface with some roughness [105], the relationship between scattering intensity and sectional area of interfacial species [100, 106] as well as the connection between sectional area of interfacial species and the amount of interfacial species [107], deduced that when concentrations of analytes in aqueous were low enough, their TIR-RLS signals were directly proportional to their concentrations. However, this quantificational basis is found by us little mentioned in the present papers on TIR-RLS [89–97].

Its optical assembly. In order to implement TIR-RLS, besides the buildings of amphiphilic species, an appropriate device should also be designed and practiced. Here, it is worthwhile to take notice of an optical arrangement, which was addressed in 1990s by Watarai et al. [108] and was placed in the sample compartment of a CFS in their in situ observations of ionic species at an aqueous/oil interface with total internal reflection fluorescence (TIRF) technique, because in our opinion, it is the original device compatible with TIR-RLS measurements as long as amphiphilic species residing at an aqueous/oil interface could emit RLS signals not fluorescence, even though at present it has not be used for this purpose. As exhibited in Fig. 6, the optical arrangement consists of two parts: (a) an optical quartz cell (10 mm) used to contain an aqueous/oil interface and (b) two right-angled quartz prisms, attached to the cell walls to make them face the excitation light source and the fluorescence detector, respectively, to allow the excitation beam to pass through the prism and the cell wall, and to impinge upon the aqueous/oil interface,

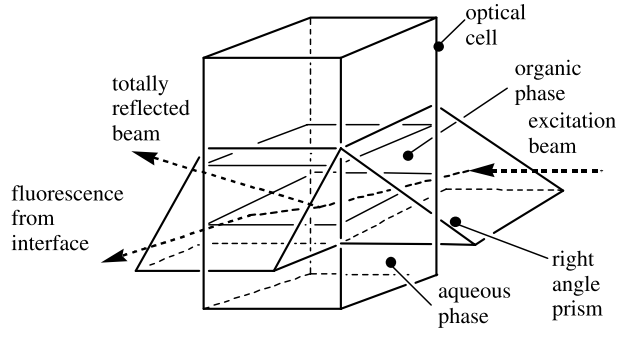

Fig. 6. Schematic drawing of the optical arrangement for TIRF measurements. Reprinted from [103] with the permission from the Japan Society for Analytical Chemistry. Copyright 1996, the Japan Society for Analytical Chemistry

and to direct TIRF signals to the detector. Besides, as described by Watarai et al. [107, 108], in order to construct a flat interface the upper inside of the cell should be treated with dichlorodimethylsilane in benzene to render it hydrophobic.

In 2000s, Huang et al. employed a device [94] for TIR-RLS detections, which, compared with the one displayed in Fig. 6, is provided with a distinct advantage that oil phase was placed under aqueous phase so as to turn away the volatilization of oil phase, surely resulting in the corresponding change in the location of the optical surfaces of the two prisms, exactly positioned not upwards but downwards.

Its advantages. According to the present papers on TIR-RLS [89–97], we consider that TIR-RLS technique is characterized by the following benefits relative to traditional RLS technique:

(I) The display of higher tolerance of coexisting foreign substances, resulting from exacting analytes from bulk phases into interfacial regions by developing amphiphilic species. As a result, TIR-RLS has the potential to directly assay real or artificial samples with highly interfering backgrounds without the dilution. As an example [89], in the assay of CTC with Eu(III) and TOPO in the presence of H_2O/CCl_4 interface, it was displayed that common ions in fluids can be allowed at high concentrations under the tolerance level of 10%, in which, especially, the tolerance concentrations of Mg(II), Pb(II), Zn(II), Al(III), and PO_4^{3-} were over 1.0×10^{-3} mol L^{-1}.

(II) The display of higher sensitivity as a result of enrichment of analytes at aqueous/oil interfaces by constructing amphiphilic species. As revealed, with RRS technique in the presence of methyl orange the limit of detection (LOD) of thiamine is 7.2 ng mL^{-1} [29], whereas with TIR-RLS technique in the presence of SDBS its LOD is 12 pg mL^{-1} [94].

(III) The display of investigating recognitions between hosts and guests with an immiscible property free from surfactants as emulsifiers, making systems more complex [89], due to the simultaneous employment of aqueous and oil phases and the encounter and interaction of these hosts and guests in interfacial regions.

(IV) The display of widely expanding interests of traditional RLS technique. TIR-RLS is a novel and effective approach to survey absorption and reaction at aqueous/oil interfaces, which are valuable in study of solvent extraction of metal ions, detection of liquid-membrane separation, ion-selective electrodes, optical sensors, and counter current chromatography [109] as well as functions of complex biomembranes [110]. Besides, the instruments necessary for TIR-RLS technique was somewhat simple and general, compared with other techniques of probing aqueous/oil interfaces in situ, such as quasi-elastic laser scattering [111, 112], second harmonic generation [113, 114] and sum frequency generation [115, 116].

Backscattering light (BSL) technique

In 2004, Huang et al. put forward a backscattering light (BSL) technique [117, 118], also termed a backward resonance light scattering (BRSL) technique [119], based on aqueous/oil interfaces.

Its basic theory. We consider the basic TIR-RLS theory above all proper described to BSL technique. However, the distinct difference between them is that the scattering angle is over 90° in BSL technique.

Its optical assembly. Compared the BSL optical assembly [117], proposed by Huang et al. for the sample chamber of a CFS, with the TIR-RLS optical assembly in their TIR-RLS studies [89–97], it is noticed by us that the important difference is the part of transmitting RLS signals from an aqueous/oil interface to the detector of a CFS, which was accomplished in the BSL optical assembly, not with a right-angled quartz prism attached to a side wall of an optical cell to face the detector of a CFS, but by attaching three quartz total reflecting prisms, plated with aluminum film on their bevels so as to lessen the energy losses as much as possible, together with a quartz cell, holding an aqueous/oil interface, placed on the top of a total reflecting prism to collect RLS signals emitted under the aqueous/oil interface. With this designed assembly, the amphiphilic species of tetraphenylporphyrin (TPP)-Al(III)-DNAs, formed via the way of interfacial interaction and thus located at H_2O/CCl_4 interface, was satisfactorily probed, of which the scattering angle was 107.4°. However, we decide that when an aqueous/oil interface is fixed, the scattering angle, which is equal to the complementary angle of the incident angle of an incident beam at an aqueous/oil oil interface, is unalterable, because the incident angle of the exciting beam is not unchangeable in this BSL

optical assembly, which, therefore, cannot offer BSL data beyond the settled scattering angle.

Soon, an improved BSL optical assembly was developed by Huang et al. [118, 119] to overcome this demerit. As compared with the former BSL optical assembly, it is noted by us that there is a wise improvement in the part of introducing an exciting beam to an aqueous/oil interface, completed by a mirror and an angle control spring instead of a right-angle quartz prism in order to acquire the exciting beam with varying incident angle at an aqueous/oil interface. As reported [118, 119], it was discovered that with the improved BSL optical assembly, increasing BSL signals was displayed until the scattering angle was increased to 154° in the study of the amphiphilic species of quercetin-cetyltrimethylammonium bromide (CTMAB), developed via the co-adsorption effect and thus adsorbed at the H_2O/CCl_4 interface. However, scattering signals should increase with the increased scattering angle ranging from 90° to 180° when other conditions were invariable [2]. As explained by Huang et al. [118, 119], due to the limitation of the device that while the scattering angle getting increased, the location of the optical cell had to be raised gradually so that the incident beam could impinge upon the H_2O/CCl_4 interface, which also made the distance between the H_2O/CCl_4 interface and the detector on the increase.

Its benefits. Compared with TIR-RLS technique, the suggested BSL is not in right-angle detection. As a result, we consider that BSL technique have the following additional advantages besides those of TIR-RLS technique:

(I) In contrast with traditional RLS technique and TIR-RLS technique performed on a CFS, BSL technique broke the permanent right-angle mode, supplying a chance to study the properties of scattered light over 90°. In addition, BSL technique is expected to show higher sensitivity because its scattering angle is in excess of 90°. For instance, with TIR-RLS technique in the presence of Eu(III) and TOPO the LOD of ctDNA is 0.16 ng mL^{-1} [91], while with BSL technique in the presence of Al(III) and TPP its LOD can reach 60 pg mL^{-1} [117].

(II) Most important, the present investigations of BSL technique are promising and valuable precursors to perceive early-warning precancerous changes and early cancerous lesions of living epithelial cells (more than 85% of all cancers originate in the epithelium, and most such lesions can be readily treated if diagnosed at an early stage [120, 121]), distinguished by the appearance and degrees of pleomorphism and hyperchromasia, , which are too difficult to detect and diagnose with common histological examination of biopsies but are exposed well in situ with a light-scattering technique without need for tissue preparation or removal in optically accessible organs [120, 121].

Corrected resonance light-scattering (CRLS) technique

Its significances. The significances of developing CRLS technique were described first by Tabak et al. [60], and then by Collings et al. [122], Li et al. [123, 124], Scolaro et al. [125], Huang et al. [126–128], and McPhie [88], respectively, which we sum up as the following:

(I) Overcoming wavelength-dependent instrumental conditions of a CFS (Instrumental adjustment). Of all published CFS-based scattering assays, ascribed to either RLS or non-RLS, most light-scattering peaks (usually maximum scattering peaks) used for analytical purposes stemmed from variational instrumental conditions in the investigated wavelength region, especially the dependences of both the intensity of the lamp and the efficiency of the detector of a CFS on wavelengths [88, 123, 124]. For example, according to our practices, with a RF-540 CFS (Shimadzu, Japan) to measure light scattering of any system, a very sharp peak will always appear near 470.0 nm, often taking on a maximum scattering peak. Furthermore, it was discovered that even for a same interacting system, RLS spectra with distinct differences could be obtained with CFSs of different models. For instance, it was reported that the light-scattering profile of polycarboxlate microspheres displayed only a light-scattering peak located at 290.0 nm when performed on a F-2500 CFS (Hitachi, Japan), but showed three light-scattering peaks, situated at 242.0 nm, 310.0 nm and 455.0 nm, respectively when performed on a F-4500 CFS (Hitachi, Japan) [126]. So, it is necessary to propose some way to solve this problem, which, we think, is a key step in judging whether a light-scattering experiment performed on a CFS is ascribed to RLS or not because a distorted light-scattering profile will without doubt mislead us.

(II) Overcoming the attenuation of light-scattering intensities as result of inner filtering of chromophores (absorption adjustment). For non-RLS systems, only when an incident beam approaching or dwelling in their absorption bands, inner filtering of chromophores should be just taken into consideration. However, for RLS systems, this effect should be always considered since RLS measurements just focus on portions of electromagnetic spectra [122]. Although preparations of extremely dilute solutions are a usual recommendation to minimize inner filtering influences, it would bring about decrease or even loss of light-scattering signals, especially when absorptions of investigated systems are comparatively high, but contents of analytes are relatively low [126]. Furthermore, for a biochemical system the dilution may often lead to various unknown changes in a few aspects, such as conformations of biomolecules and degrees of association [127]. Thus, it is also compulsory to put forward some approach to treat with this problem.

Its approaches. Of all reported CRLS [60, 88, 122–128], we consider that it is worthwhile to pay attention to an approach, suggested by Tabak et al. [60] in 1997, in which the discussions of instrumental adjustment and absorption adjustment are both involved, because compared with other approaches [88, 122, 125], such as the one brought forward by Collings et al. [122] in 1999, which is in need of exact knowledge of the structure factor of scatterers and also has to take polarization effects into account, this approach is fairly simple and also effective, therefore absorbing more attentions from other researchers, such as Li et al. [123, 124] and Huang et al. [126–128], and afterwards further developed especially by Huang et al. [126–128]. Herein, we first talk over instrument adjustment and absorption adjustment, respectively, and its impact on scattering signals, and then take them into holistic consideration.

(I) Instrumental adjustment. Here, the interacting system of proteins with sulfonazo III [123] is used as an example in taking about the instrumental adjustment approach.

According to Rayleigh's law, I_{sca} is in direct proportion to ${\lambda_0}^{-4}$ when other parameters keep constant, shown in Eq. (5). So, measured I_{sca} of pure water, $I_w(\lambda_0)$, should obey this law. However, it was found by Li et al. [123] that I_w in the scanned range from 250.0 to 700.0 nm did not obey this law, which was considered due to the dependence of the used CFS sensitivity on λ_0. Therefore, it is wise of them to employ a CFS sensitivity, $S(\lambda_0)$, first recommended by Tabak et al. [60] and defined as

$$S(\lambda_0) = I_w(\lambda_0)/R_w(\lambda_0) \tag{13}$$

in which $R_w(\lambda_0)$ is the Rayleigh spectrum of pure water normalized to unity with the value of $I_w(\lambda_0)$ at 250.0 nm, namely,

$$R_w(\lambda_0) = (250/\lambda_0)^4 I_w(250.0\,\text{nm}) \tag{14}$$

Obviously, the value of $S(\lambda_0)$ comes up to 1 only when the I_0 at any λ_0 keeps invariable, which cannot be achieved with any CFS, so $S(\lambda_0)$ is an effective parameter to describe variational sensitivity of a CFS at any λ_0. With $S(\lambda_0)$, corrected light-scattering intensity, $I_{corr}(\lambda_0)$, could be thus obtained with measured light-scattering intensity, $I_{CFS}(\lambda_0)$, in the light of the following expression [123]:

$$I_{corr}(\lambda_0) = I_{CFS}(\lambda_0)/S(\lambda_0) \tag{15}$$

With Eq. (19), it was uncovered that the corrected light-scattering spectra of both sulfonazo III and its interaction with BSA followed Rayleigh's law well in whole investigated wavelength range from 250.0 to 700.0 nm, verifying no exceptional increase of $I_{corr}(\lambda_0)$ in the absorption region of either sulfonazo III or sulfonazo III-BSA complex, and as a result, this light-scattering experiment was not ascribed to RLS. However, it is a pity that the investigations of Li et al. [123, 124] ignores corresponding adsorption adjustments, later proved to show noticeable influences on detection sensitivities [126–128].

In our opinion, this instrumental adjustment approach availably makes up for wavelength-dependent instrumental conditions of a CFS, as above mentioned, and as a consequence recuperates thus-denatured light-scattering signals, which casts off various light-scattering peaks, just resulting from the unequal instrumental conditions, and therefore is important in estimating whether enhanced light-scattering signals, displayed by a CFS, issue from RLS effect or not. Besides, with the proposed approach, spectral discrepancies of a same investigated system, displayed on CFSs of different models, can be corrected as well by a sensitivity conversion factor, i.e., a ratio of sensitivities of used CFSs, acquired according to Eq. (13).

(II) Adsorption adjustment. As we discuss above, that RRS [43–51] prior to 1993 could not attain signals, measured by a CFS, is as a result of

slightly enhanced scattering signals swallowed by absorption of chromophores. Although, it is sapiential of Pasternack et al. [3–6] to magnify signals as much as expediently detected with a CFS, absorption of chromophores still wears down scattering signals. Accordingly, adsorption adjustment is also necessary, which is beneficial to increase detection sensitivity.

Starting with 2002, Huang et al. [126–128] have employed an improved approach, in contrast with the one put forward by Tabak et al. [60], to manage absorption adjustment only with a CFS. However, these approaches both include two basic considerations: one, coping with decreased I_0, and the other, handling with lessened I_{sca}. We cover their main ideas as the following:

For a minute volume unit of $dxdyZ_0$ with a distance of x from lamp, as an incident light intensity at any λ_0 decreases from I_0 (λ_0) to $I_x(\lambda_0)$ as a result of passing through such a distance in a colored sample solution, the scattering intensity of the minute volume unit, $d(I_{sca}(\lambda_0))$, can be expressed as

$$d(I_{sca}(\lambda_0)) = \beta I_x(\lambda_0) Z_0 dxdy \tag{16}$$

where β was a coefficient of proportionality, dx and dy are partial differentials in the directions of the exciting beam and the scattered beam, respectively, and Z_0 is the height of the exciting beam passing through the sample solution. In accordance with Lambert-Beer Law, when the exciting beam going through an x distance of the sample solution and the whole sample in the cuvette with a path length of b, correspondingly, I_0 (λ_0) decreases to $I_x(\lambda_0)$ and $I_t(\lambda_0)$, respectively, then

$$\log \frac{I_x(\lambda_0)}{I_0(\lambda_0)} = -axc \tag{17}$$

and

$$\log \frac{I_t(\lambda_0)}{I_0(\lambda_0)} = -abc \tag{18}$$

in which a was the constant in the concept of Lambert-Beer Law. Consequently, it is easy to obtain

$$I_x(\lambda_0) = I_0(\lambda_0) T(\lambda_0)^{x/b} \tag{19}$$

in which $T(\lambda_0)$ is the transmittance with the value of $I_t(\lambda_0)/I_0(\lambda_0)$. Then, according to Eqs. (20) and (23), the following expression can be acquired,

$$d(I_{sca}(\lambda_0)) = \beta I_0(\lambda_0) T(\lambda_0)^{x/b} Z_0 dxdy \tag{20}$$

Also, since the signal of $d(I_{sca}(\lambda_0))$ needs to cross a distance of y in the colored sample solution before going into a detector, similar to Eqs. (16) and (19), the actually measured scattering intensity, $d(I'_{CFS}(\lambda_0))$, can thus be given as

$$d\left(I'_{CFS}(\lambda_0)\right) = \beta I_0(\lambda_0) T(\lambda_0)^{x/b} T(\lambda_0)^{y/b} Z_0 dxdy \tag{21}$$

Here, we should emphasize that Eqs. (17) to (21) characterize the dissimilarity of CRLS approaches, set out by Tabak et al. [60] and Huang et al. [126–128], respectively. In the approach of Tabak et al. [60], $A(\lambda_0)$, instead of $T(\lambda_0)$, of the sample solution is used to express the influences of adsorption on intensities of both incident and scattered beams. According to Huang et al. [126–128], an absorption holder, an accessory equipped with some models of CFSs, such as F-2500 or 4500 (Hitachi, Japan), could be taken up to measure T without the assistant of a spectrophotometer, which could turn away errors, deriving from their different instrumental conditions, as much as possible besides the corresponding convenience.

According to Eq. (21), supposing that absorption of chromophores has been compensated completely, the corrected scattering intensity, I'_{corr} (λ_0), can be acquired as the following,

$$d(I'_{corr}(\lambda_0)) = \beta I_0(\lambda_0) Z_0 dxdy \tag{22}$$

If X_0 and Y_0 represent the final distances of incident and scattered beams going through the sample solution, then the expression of I'_{corr} (λ_0) can be given as

$$\begin{aligned} I'_{corr}(\lambda_0) &= \beta I_0(\lambda) Z_0 \int_0^{X_0} \int_0^{Y_0} dxdy \\ &= \beta I_0(\lambda) Z_0 X_0 Y_0 \end{aligned} \tag{23}$$

Also the measured scattering intensity, I'_{CFS} (λ_0), is obtained,

$$\begin{aligned} I'_{CFS}(\lambda_0) &= \beta I_0(\lambda) Z_0 \int_0^{X_0} T(\lambda_0)^{x/b} dx \int_0^{Y_0} T(\lambda_0)^{y/b} dy \\ &= \beta I_0(\lambda) Z_0 b^2 \frac{[1 - T(\lambda_0)^{X_0/b}][1 - T(\lambda_0)^{Y_0/b}]}{[\mathrm{In}\, T(\lambda_0)]^2} \end{aligned} \tag{24}$$

For an often used 1.0 cm cuvette, when $X_0 = Y_0 = b = 1$, then

$$\begin{aligned} I'_{CFS}(\lambda_0) &= \left[\frac{1 - T(\lambda_0)}{\mathrm{In}\, T(\lambda_0)}\right]^2 \\ I'_{corr}(\lambda_0) &= F_{com}(\lambda_0) I'_{corr}(\lambda_0) \end{aligned} \tag{25}$$

in which $F_{com}(\lambda_0)$ is a correcting factor in the such condition. As revealed, with this adjustment approach, the detection sensitivities of proteins with either Fast

Red VR (FRV) [127] or Ponceau G (PG) [128] could all be increased about two folds, and these sensitivity enhancement effects displayed much more obvious with the concentration of FRV or PG increased. However, Huang et al. [127, 128] neglect unequal instrumental conditions, often resulting in peaks beyond essences of investigated systems [60, 88, 123, 124].

Obviously, adjustments of instrument and absorption can be easily taken into integral consideration, combining Eq. (24) or (25) with Eq. (15). However, according to us, to some extent we consider instrumental adjustment more important and interesting because it is valuable to help us to distinguish RLS from other scatterings.

Resonance light-scattering imaging (RLSI) technique

Its intentions. As reported [42, 80], the establishment of RLSI technique is rooted in two considerations at least. One is that it is aimed to break through the limitation of detecting average RLS features of scatterers in bulk solutions or at aqueous/oil interfaces, which fails to directly observe a single scatterer. The other is that most importantly, its final goal is to present a new biomedical imaging technique for managing problems of cells, tissues and organs so as to put disease diagnosis into practice, which is expected to be advantageous compared with frequently used diagnostic imaging techniques, such as high energy X-rays and expensive nuclear magnetic resonance.

Its approaches. As considered by us, at present there are two representative approaches to handle RLSI: One, proposed by Yguerabide et al. [2, 31] and the other, planed by Huang et al. [80, 129]. We should elucidate that the approach from Yguerabide et al. [2, 31] was first published in 1998, but until 2002 it just began to be employed by other researchers [24, 27], and besides, it is of much interest to compare it with the one from Huang et al. [80, 129], which is a complement to it. Therefore, the approach contributed by Yguerabide et al. [2, 31] is also here discussed. According to us, the fundamental discriminations of these two approaches are based on the following aspects:

(I) Using different probes, which, considered by us, is the most essential distinction between them because their other distinctions develop from it. For the approach from Yguerabide et al. [2, 31], RLS Particles^{TM} is focused on, but for the approach from Huang et al. [80, 129], SOMs are interestingly investigated.

(II) Using different instruments, resulting from the extremely disparate light-scattering power between RLS Particles^{TM} and SOMs. A 60-nm gold particle, for example, with regard to light-emitting power, was equivalent to about 3×10^5 fluorescein molecules, which cannot be achieved at all with SOMs [2]. Therefore, relatively simple instruments can be put into use in the approach from Yguerabide et al. [2, 31, 79], in which two instruments are typically utilized. For liquid phase homogenous imaging assays, a standard scanner with a charge-coupled device (CCD) camera is as much as necessary. Here, it is necessary to talk of a scanner developed by Genicon Sciences, named GSD-501^{TM} RLS detection and imaging instrument, in which a 10 W metal halide arc lamp is satisfactory used as an illuminator. However, in the approach from Huang et al. [80, 129], an Olympus IX70 inverted microscope equipped with a CCD camera and an argon ion laser source are required. For imaging applications in cell biology, histology, microarrays and microfluidics, a simple student microscope armed with a 100 W filament lamp and low magnification objective and ocular ($\times$100 total magnification) for observation or/and a CCD camera for capturing images. At present, we cannot locate the corresponding imaging applications of the approach from Huang et al. [80, 129] on the fields mentioned above except microfluidics, in which, in like manner an Olympus IX70 inverted microscope equipped with a CCD camera and an argon ion laser source are all necessary.

(III) Used for different occasions. For the approach from Huang et al. [80, 129], first, it is not convenient to be employed in the occasions that systems are labeled with SOMs, which are prone to photobleaching or characteristic scattering peaks of systems are far away from 488.0 nm as a consequence of very strong power of the laser source and its exciting wavelength rooted at 488.0 nm, respectively. Second, the approach from Huang et al. [80, 129] is specifically designed to investigate behaviors of self-aggregations of SOMs or their self-assemblies on analytes, which interacts with themselves or analytes mainly with

electrostatic force or/and hydrophobic force, which is easy to be disturbed by foreign coexisting substances and thus is only fit for relatively simplex systems. However, for the approach from Yguerabide et al. [2, 31, 79], the case is just opposite. As stated above, RLS Particles^{TM} can produce very stable and strong scattered light, which can be measured with an instrument free of a laser source and can be permanently archived. Moreover, the scattering wavelengths of RLS Particles^{TM} can be tuned via various well-suited methods for a purpose of advantageous detection or many other purposes. Evenly importantly, RLS Particles^{TM} can be easily to make functional ones, which supports specific binding. Due to these factors, RLS Particles can be with no trouble used in both simplex and complex systems.

Flow injection resonance light-scattering (FI-RLS) technique

Its Establishment and Benefits. Flow injection (FI), as a powerful technique of automatically inputting samples and providing an format to manage systems without compulsorily reaching physical/chemical equilibrium, has been being effectively coupled to lots of optical and electrochemical analysis and detection modes, such as spectrophotometry [133], fluorometry [134], infrared spectrophotometry [135] chemiluminescence [136], atom absorption/emission spectrophotometry [137, 138] and electrochemiluminescence [139]. Surely, the appearance of RLS technique [3–6] affords a novel and expecting combining prospect to it. However, until 2003, conventional Rayleigh scattering technique based on a CFS was just first combined with it to actualize a light-scattering on-line detection of total proteins with high sample throughput [140]. Not RRS but conventional Rayleigh scattering occurred [140], but we believe that their attempt do inaugurate a new application format for RLS technique. Beginning from 2004, Huang et al. [141–143] have been showing interest in developing and applying FI-RLS technique, disclosing that it could provide RLS technique with a few extra merits, such as well improved reproducibility, obviously decreased consuming time and effectively reduced amounts of samples, and interestingly superinduced applicability for studying relatively unstable systems.

Selecting flow injection (FI) configurations. According to Fernández Band et al. [140], in order to select an appropriate FI configuration for an RLS work, the following basic aspects should be considered: (I) obtaining a stable baseline, (II) obtaining signals with good sensitivity and (III) obtaining signals with good reproducibility. In the light of these aspects, several configurations were tested [140] in the determination of total protein in urine and serum samples with bromothymol blue (BB) and it was found that the configuration, displayed in Fig. 7 and characterized by injecting an analyte, carried by a buffer, into the solution of the buffer and a label, well mixed in advance in R_1, and making them well contact and interact in R_2, was most satisfactory. We believe that this configuration is a representative one suitable for systems, in which labels interact with analytes mainly with electrostatic force. As for the FI-RLS configurations proposed by Huang et al. [141–143], we note that a light emitting diode (LED), a light source extensively employed in absorbance, fluorescence and spectroelectrochemical measurements [144], in place of xenon lamp, regularly utilized as a light source in RLS detections, is interestingly used as a exciting source. As explained [141], in contrast to an xenon lamp, first, an LED is a cooler light source in its all working wavelengths, which is consequently more advantageous to observe systems with photosensitive labels.

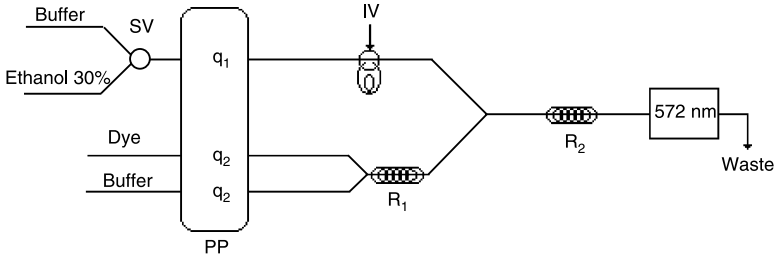

Fig. 7. FIA system for the determination of total proteins. *PP* Peristaltic pump, q_1 1.35 mL min^{-1}, q_2 0.68 mL min^{-1}, *SV* selection valve, *IV* injection valve, $R_1 = R_2 = 600$ mm. Reprinted from [140] with the permission from Springer Science and Business Media. Copyright 2003, Springer Science and Business Media

Second, a LED is also provided with longer life and lower cost.

The present of resonance light-scattering (RLS) technique in analytical chemistry (2005–2006)

Wavelength-ratiometric resonance light-scattering (WRRLS) technique

As shown in Fig. 2, the appearance of a novel RLS derived technique, wavelength-ratiometric resonance light-scattering (WRRLS) technique, characterizes the progress of traditional RLS technique in analytical chemistry from 2005 to the present. Here, we should first clarify that Lakowicz et al. [151] first proposed WRRLS technique in 2003 and in 2005 with this technique they probed glucose [75] with fascinating benefits. Furthermore, very soon Huang et al. [130, 145] began to pay attention to this technique but made their distinguishing studies. So, we believe that it is of interest to talk about the contributions from Lakowicz et al. [75, 151] and Huang et al. [130, 145] together here.

The purpose of its establishment

With more and more analytical practices of traditional RLS technique, it has been found that especially when SOMs in place of RLS Particles^{TM} are used as RLS labels, RLS detections suffer from difficultly self-restrained signal fluctuation [42, 130, 145, 146] more or less, deriving from a variety of poorly quantified variable factors, such as the incident light intensity, real reagent concentration, and medium conditions, including pH, ionic strength, temperature and polarity, as a result of being confined to single-wavelength measurements, directly using data from instruments. Furthermore, due to signal fluctuation [146], it is inconvenient with traditional RLS technique to collect real time signals of investigated systems for dynamics studies. Consequently, it is necessary to develop a proper way to resolve this problem.

Its establishment and achievement

Similar to spectrofluorimetry in wavelength-ratiometric format, first proposed and used in 1985 by Grynkiewicz et al. [147] in biochemical studies of the physiological role of cytosolic free Ca^{2+} with a new family of synthesized highly fluorescent indicators, and later widely employed in various assays, such as monitoring amines [148], molecular oxygen [149] and magnesium ion [150] inside viable cells, and proved a satisfactory approach to compensate for the variable factors above mentioned with no direct use of data displayed by CFSs but corresponding data ratios, the development of RLS technique in wavelength-ratiometric format is surely of interest and importance [130, 145, 146].

The contribution from Lakowicz et al. As early as in 2003, Lakowicz et al. [151] first put forward a new optical sensing approach based on wavelength-ratiometric gold or silver colloid (i.e., RLS particles^{TM}) scattering, which, as above described, is in essence ascribed to WRRLS, and exposed that ratios of light scattered at two incident wavelengths could be verified to reliably analyze the extent of colloid aggregation, which were independent of the total colloid concentration over a wide range of colloid concentrations, indicating that this approach got the potential in a wide variety of bioaffinity assays, such as the good promise to sense glucose based on gold or silver colloid aggregation induced by dextran and concanavalin A (Con A). Moreover, Lakowicz et al. [151] showed the basic principle to select two proper incident wavelengths for wavelength-ratiometric measurements, which was rooted in an absorption ratiometric curve, acquired by dividing an absorption curve of an analyte-label conjugation with that of only corresponding label, and in which, in general, maximum and minimum peaks should be considered, resulting in an obvious ratio. According to us, this principle well reflects the close relationship between absorption and RLS. In addition, it is worthy of being noted that Lakowicz et al. [151] suggested a device with potential simplicity for WRRLS in the presence of gold or silver colloid, mainly composed of a white LED, two filters for different wavelengths and two solid state detectors all with orthogonal geometry to the white LED, and that as stated by them [151], it was benefit to develop such a device for medical sensing with WRRLS technique. In 2005, by virtue of WRRLS technique performed on a self-developed, simple and low-cost experimental setup primarily made up of a white LED and a simple scanning monochromator placed with a orthogonal geometry, Lakowicz et al. [75] established a simple glucose sensing platform in the presence of dextran-coated gold colloids and Con A, actualizing the above-mentioned promise and disclosed that by measuring the ratios of scattered light intensities at two different arbitrary wavelengths,

560 and 680 nm, glucose concentrations could be readily detected from a few millimolar up to ~60 mM and that this approach to glucose sensing, coupled with the sensing aggregates' ability to scatter red light, displayed its potential use in physiological transdermal glucose monitoring, either for implantable skin sensors or glucose sensing tattoos. Equally important, Lakowicz et al. [151] presented several benefits of WRRLS technique in the presence of gold or silver colloid in contrast with traditional colloidal plasmon absorption, in which, as considered by us, except casting off signal fluctuation, benefiting from an wavelength-ratiometric format, other benefits all develop from the advantages of RLS Particles™ in detail above described, such as developing very simple and low-cost instrumentation (very powerful light-scattering ability of RLS Particles™) and adjustable LOD (easiness to acquire RLS Particles™ with different diameters). We believe that the works of Lakowicz et al. [75, 151] enrich RLS application formats as well as further exhibit interests of RLS Particles™ in RLS studies, which, as a result, are good complements to those of Yguerabide et al. [2, 31, 79].

The contribution from Huang et al. After the introduction and applications of WRRLS technique from Lakowicz et al. [75, 151], Huang et al. soon also used this technique, termed resonance light scattering ratiometry (RLSR) technique by them, in their analytical practices [130, 145]. Compared with these investigations of Lakowicz et al. [75, 151], those of Huang et al., as considered by us, are distinguished by focusing on labels of SOMs and describing the benefits of RLSR in contrast to traditional RLS technique based on SOMs.

In 2005, Huang et al. [130, 145] put RLSR technique into practice, using the interaction of a typical biopolymer medicine, heparin (HP), with Janus Green Blue (JGB) [130], TAPP [145] and *meso*-tetra (4-methylpyridy) porphyrin (TMPyP-4) [145], separately, as model systems, during which it was found that RLSR technique was provided with a few following benefits:

(I) RLSR technique supports a more efficient way to make use of RLS signals in simultaneous consideration of the features of corresponding absorption spectra, which is displayed in selecting detection wavelengths for measurements. It is necessary and important because RLS is an exceptional light-scattering phenomenon characterized by great enhancement of scattered light in the case of close proximity to an absorption band of extended chromophores with good electronic coupling [3–6]. According to Huang et al. [130, 145], in general, the principle of selecting detection wavelengths for RLSR studies is based on peaks and valleys of UV-visible spectral ratio curves, which can be obtained by dividing UV-visible spectra of the scattering systems composed of RLS labels and analytes with those of RLS labels and thus accords with the corresponding principle proposed by Lakowicz et al. [75, 151]. In our opinion, this term is also propitious for us to distinguish RLS ones for non-RLS ones.

(II) RLSR technique exhibits much better tolerance ability to dubious variations of environmental conditions mentioned above [130, 145], displaying that it gets superior ability in signal stability and thus is suitable for dynamic studies. As tested by Huang et al. [130], for the investigated system of HP binding with JGB, RLS ratios remained constant over a wide pH range of 2.0–9.9, while the RLS signals obtained from a single-wavelength format stayed constant only in a narrow range of 7.0–9.9. Furthermore, it was found that RLSR technique can be more accurate and intuitionist in dynamic investigations. By way of example, as has been proved by Pang [141], when the concentration of TAPP was kept at $1.0\,\mu g\,mL^{-1}$ and that of HP was gradually increased from $0.84\times10^{-6}\,mol\,L^{-1}$ to $1.68\times10^{-6}\,mol\,L^{-1}$, with traditional RLS technique RLS signals at 418.0 nm, a characteristic valley, decreased and those at 448.0 nm, a characteristic peak, nearly had no changes, not clearly displaying the dynamic process of the interaction of TAPP with HP, whereas with RLSR technique it was not the case since it was found that the corresponding RLS ratios got increased steadily.

(III) RLSR technique makes better-quality analytical performances available. Unlike traditional RLS technique, RLS ratio decreases exponentially with increasing concentrations of analytes, but has a perfect linear relationship with the logarithm of concentrations of analytes in wide ranges, as showed as the following:

$$I_{\text{ratio}} = a - b' \log c \tag{26}$$

in which c is concentrations of analytes, a and b' are both constants, varying with investigated systems when instrumental conditions are fixed. In view of this fact, the LODs of RLSR technique have the promise to be much better than those of traditional RLS technique when other conditions are uniform, such as investigated systems and instrumental conditions. Accordingly, RLSR technique is of much advantage to probe analytes with relatively low concentrations. As practised by Huang et al. [130], for the system of heparin interacting with JGB, when a Hitachi F-2500 spectrofluorometer (Tokyo, Japan) was put into use and the concentration of Janus Green Blue was 1.0×10^{-5} M, with traditional RLS technique the LOD was 8.87 ng L^{-1} (3σ) while with RLSR technique the LOD was as low as 0.03 ng L^{-1} (3σ).

In addition, according to us it is worth noticing that during investigating the enhanced light-scattering nature of the interaction of JGB with HP [130], a submicron particle size analyzer was first and smartly introduced to monitor size distributions of light-scattering particles in solutions based on photon correlation spectroscopy, which in practice proved advantageous to with data account for the contributions of sizes and numbers of scatterers to enhanced RLS signals. As revealed by Huang et al. [130], when the concentration of JGB remained unchanged, as an example, at 1.0×10^{-5} mol L^{-1} and that of heparin was on the increase in an appropriate range, there were relatively both smaller and larger scatterers in the investigated system, making various contributions to the enhanced RLS signals. To be exact, in the presence of 100 ng L^{-1} heparin, the contribution of the relatively smaller particles with 248.1 nm in size to the enhanced RLS signals was 48%, whereas that of the relatively larger ones with 2913.5 nm in size to the enhanced RLS signals was 52%. However, when the heparin content was increased to 300, and 500 ng L^{-1}, separately, the sizes of relatively smaller particles decreased from 248.1 to 177.1 and 161.8 nm, respectively, and their contributions to enhanced RLS signals got decreased from 48% to 20% and 17%, respectively, while those of relatively larger ones changes from 2913.5 to 1692.9 and 1363.6 nm, correspondingly and their contributions to enhanced RLS signals increased from 52% to 80% and 83%, respectively, which further proved when the content of JGB was constant, with the increase of heparin such interacting scatterers got smaller in size and the number of relatively smaller scatterers tended to drop off, but that of relatively larger scatterers had a tendency to get increased. Therefore, Huang et al. concluded that either the enhancement in granularity of scatterers or the development in number of scatterers would lead to the enhanced RLS signals.

At last, representative contributions on RLS derived technique from 2001 to 2006 are concluded in Table 3.

Table 3. The representative contributions on derived resonance light scattering (RLS) techniques from 2001 to 2006, including those ascribed to conventional Rayleigh scattering or large particle light scattering

No.	Name	Authors	Time	Novelty	Ref.
1	TIR-RLS	Huang et al.	2001	1. Developing a method to assay analytes at liquid/liquid interfaces 2. Proposing advantages of detect analytes at liquid/liquid interfaces 3. Bring forward a wise device for TIR-RLS measurements	[89]
2	WRRLS	Lakowicz et al.	2003	Actualizing wavelength-ratiometric gold or silver colloid scattering	[151]
3	CRLS	Huang et al.	2003	Setting out an effective way to compensate for the molecular absorption decreasing RLS signals with an absorption device attached to a conventional fluorescence spectrophotometer	[127]
4	RLSI	Huang et al.	2003	Suggesting a way to survey imaging of scatterers without RLS Particles™	[80]
5	FI-Rayleigh scattering	Fernández Band et al.	2003	Coupling FI with Rayleigh scattering technique	[140]
6	BSL	Huang et al.	2004	Putting forward a device to conduct BSL at liquid/liquid interfaces	[117]
7	WRRLS	Lakowicz et al.	2005	Carrying out nanogold plasmon resonance-based glucose sensing with wavelength-ratiometric RLS	[152]
8	RLSR	Huang et al.	2005	Performing RLSR without RLS Particles™	[130]

The future of resonance light-scattering (RLS) technique in analytical chemistry (2006–)

To further its theory

Resembling well known fluorescence, RLS is also a light radiation in the presence of an incident light, bringing about the essence that if only RLS signals are powerful enough, they can be satisfactorily measured with a CFS. However, at present RLS theory is not as satisfying as that of fluorescence because as considered by us, it at least has not yet expound RLS phenomena well with a quantum mechanical point of view, i.e. with transition of electron between energy levels of self-aggregations or self-assemblies of chromophores, which is surely more helpful to further disclose RLS phenomena in nature compared with the present discussions on RLS theory talked above. To further RLS theory with a viewpoint of quantum chemistry, as thought by us, the following basic problem should be clarified:

(I) How energy levels, radiating RLS, form in the process of self-aggregations or self-assemblies of chromophores and what structural factors are positive ones during this process, which will be much favored to be solved with establishing a quantitative structure-property relationship with methods of quantum chemistry and will in essence guide the application fields of RLS and the selections of RLS labels. As conformed by Pasternack et al. [3–6], synchronous scan spectra of both porphyrins and chlorophyll *a* in the form of monomer and aggregation, respectively, have very different spectral characteristics (mainly shown in the locations of maximum peaks), indicating, according to us, that there are two dissimilar types of energy levels for monomers and aggregations. Although Jiang suggested that a supramolecular interface energy band (SIEB) could be developed in self-aggregations or self-assemblies of chromophores to explain RLS [46], but in our opinion it is lack of a general and confirmed consideration to elucidate how an SIEB forms and what factors are basic ones in the form of an SIEB, generating RLS. Besides, because doubling scattering ($\lambda_{ex} = 1/2\lambda_{em}$) and anti-doubling scattering ($\lambda_{ex} = 2\lambda_{em}$), were discovered by Liu et al. [16] to go always with RRS, and were later termed as second-order scattering (SOS) and frequency doubling scattering (FDS) [17] by them, respectively, it is necessary to set out an explanation to this phenomenon as well.

(II) What the differences exist among energy levels producing RLS signals and those emitting other signals, such as above described conventional Rayleigh scattering and large particle scattering, often and easily mistreated as RLS, and fluorescence, often discovered to coexisting with RLS [11, 56]. To do this work will result in the following benefits at least:

  (a) Making good use of techniques of conventional Rayleigh scattering and large particle scattering, also regarded as ''derived'' techniques of RLS, which have been validated to be feasible ways to deal with colorless or non-fluorescigenic samples with obvious enhancement in particle size [37–40].
  (b) Making a good distinction between fluorescence, especially resonance fluorescence, and RLS.

In addition, in exploring the causes of enhanced RLS signals, a particle size analyzer and an atom force microscope (AFM) will exhibit their interesting contributions.

To further its analytical applications

Although RLS technique has been applied in analytical chemistry for over a decade, in which a few RLS derived techniques have been set up and applied, we are still considering that there are great application potentials of RLS technique in analytical chemistry. As believed by us, in future at least the following aspects are worth anticipating:

(I) Developing platforms of heterogeneous RLS assays, especially with high throughput analyses. As described above [31, 79], RLS technique is compatible with numerous analytical platforms of both homogeneous and heterogeneous assays. However, most of the present RLS analytical applications are confined to time-consuming homogeneous assays in solution phases. Therefore, it is of much interest to develop RLS applications in various analytical platforms of heterogeneous assays, especially with high throughput analyses. For example, RLS technique can combine with microarray technique,

which is an extremely important research tool for biochemistry and can be addressed on a variety of supports [152], such as planar surfaces, microchannels and microwells, to enable the massively parallel detection of a wide range of biomolecules with high throughput, such as proteins (antigen, antibodies, enzymes), oligonucleotides, nucleic acids, lipids and carbohydrates, for monitoring, diagnostic and treatment purposes. The development of a microarray-based RLS technique, we believe, is of great significances for analytical chemistry in many aspects, such as in pioneering a powerful way to unravel functions of hundreds of thousands of genes in a massively fashion in the post genomic era, showing valuable to achieve personalized medicines in future and screening combinatory libraries for leading drug discoveries.

(II) Developing an immuno-RLS technique. As reported [31, 79], one of the important applications of RLS technique in analytical chemistry is to sense a variety of biochemical contents with a bioaffinity format. However, at present in most of these practices, bioaffinity are simply based on electrostatic force and hydrophobic force between targets and SOMs [8, 9, 11, 18–22, 25, 26, 28–30, 33–36], which will surely not impossible to secure a specific bioaffinity in a complicated biochemical event, such as an event in cytology and histology. Therefore, it is necessary to develop an immuno-RLS technique, in which bioaffinity between targets and labels are carried out via specific antigen-antibody associations. The establishment of an immuno-RLS technique will largely advance RLS applications in clinical, pharmaceutical and environmental chemistry, in which RLS Particles^{TM} will be definitely display their charming functions due to their advantages discussed above.

(III) Developing an angle-scanned RLS technique. It is well known that light-scattering signals are of angle-dependence [1]. Nevertheless, currently most RLS detections are rooted in orthogonal geometry, leave out studies of characteristics of RLS signals deviated from 90°C, producing two RLS signals, i.e., forward RLS signals and backward RLS signals, and their analytical applications. Although, Huang et al. have made their attempt in this aspect, their work do not involve the corresponding studies of forward RLS signals and furthermore, their self-developed device is limited to observe light-scattering signals beyond 154°. Therefore, it is of much interest to develop an instrument to achieve angle-scanned RLS technique to obtain complete spatial distribution of RLS signals, which is sure to provide a good opportunity for analysts to choose with what degree to acquire advantageous RLS signals for their purposes. For example, RLS measurements in backscattering geometries will open the possibility of working with nearly opaque samples or thin films, which is useful to probe states of aggregations of chromophores in organelles of cells or of aggregates in sensor arrays [6, 120, 121]. Besides, with an angle-scanned RLS technique angular-ratiometric RLS signals can be conveniently obtained and used for bioaffinity sensing.

(IV) Developing various incorporated RLS techniques. RLS technique, serving as a powerful signal generation and detection platform, can interestingly be incorporated with many other engrossing techniques, for typical examples, capillary electrophoresis (CE) and chemical cytometry (CC), to remarkably enhance its impacts and functions in analytical chemistry.

CE, which, in clinical and biological assays, is an important and frequently used separation technique and furthermore is of great promise to replace many conventional separation techniques, especially electrophoresis and high performance liquid chromatography (HPLC), as a result of many merits, such as consuming small amounts of sample and being able to separate large or small analytes fast and well with either neutral or charged property [153], can couple with RLS technique to bring about two appealing profits. That is, for RLS technique, CE can professionally solve great interferences of coexisting foreign substances in complicated samples before RLS detections thus to largely expand its application events, and for CE, compared with its present detection approaches on the top for use, such as indirect or direct absorption and conductometric detection [154], the introduction of RLS technique is believed to provide a sensitive readout method to it.

CC refers to the automated measurements of oligonucleotides, small molecules, proteins and enzyme activity in single cells, which is impor-

tant in clinical and biological science, and can be performed with either imaging with a fluorescence microscope or characterizations with spectroscopic and electrical means [155]. With Immuno-RLS Particles™, RLS and RLSI technique are in no doubt to display their captivating roles in CC, which is superior to fluorescence spectrophotometry and imaging technique as a result of the characteristics of RLS Particles™ talked over above.

(V) Developing microfabricated analytical devices, also referred to as lab-on-a-chip or micro total analysis systems (μ-TAS), for RLS detections. At present, one of the trends of the developments of laboratory analytical devices is to manufacture microfabricated analytical devices to make them become smaller, simpler, and smarter [156], which are characterized by improved performance, increased speed (high throughput), reduced costs and reagents consumption and the proposed possibility of parallel and integrated analyses and thus are more convenient and powerful in dealing with clinical and biological events [157]. For a typical example, the development and implementation of RLS devices combined with microfluidic technique, involving both established and evolving technologies, including microlithography, micromachining, micro-electromechanical systems technology and nanotechnology [157], is about to fulfill these new requirements in RLS analytical applications, which will urge RLS technique to be favorably employed in pop fields in analytical chemistry, such as in rapidly developing fields of genomics, proteomics and metabolomics and in aiding in diseases diagnostics, drug discovery and evaluation of new pharmaceuticals.

(VI) Developing RLS detections in infrared absorption envelopes. To date, RLS detections are restricted to UV-Vis absorption envelopes, not yet treating of infrared absorption ones. However, many compounds have infrared absorption bands. As an example, cyanine dyes bears the characteristic of large molar absorbability, the spectra of which can be extended from visible region to near infrared (near-IR) by tuning the length of the conjugate chain [158]. Furthermore, it is promising to efficiently decrease background interferences to develop RLS detections in infrared absorption envelopes.

Conclusions

In this paper, we try to review major achievements of RLS technique in analytical chemistry from 1995 to the present, involving those of its various derived techniques, based on selected peer-reviewed published papers and dissertations.

In our opinion, although RLS technique has been applied in analytical chemistry for over 10 years and so many papers have been published in most international journals for analytical chemistry, not only its theory but also its analytical applications are necessary and expected to be advanced further to more greatly display its functions in analytical chemistry. We believe that the developments of RLS technique in analytical chemistry are of great potential, interest and anticipation.

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