Title	Single Nanoparticle Tracking at the Liquid/Liquid Interface by Total Internal Reflection Fluorescence Microscope
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Citation	大阪大学, 2019, 博士論文
Version Type	
URL	https://hdl.handle.net/11094/73517
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Form 3

Abstract of Thesis

Name (Gong Wanyun)	
Title	Single Nanoparticle Tracking at the Liquid/Liquid Interface by Total Internal Reflection Fluorescence Microscope （全内部反射蛍光顕微法を用いた液／液界面における単一ナノ粒子の動きの追跡）

Abstract of Thesis

The liquid/liquid interface generated by two immiscible solvents plays a dominant role in modern science and technology due to its extensive applications in industry[1], medicine[2], biology[3], and microemulsions[4]. Despite the liquid/liquid interface has great importance for modern science and arise many scientific attentions, it is still facing many challenges especially on the measurement of the physical and chemical properties of interface.

Interfacial viscosity is a basic physical property, which has a more practical significance in revealing mechanism many topics, such as fractal and dendritic interface growth[5], phase transitions in adsorbed interface[6] as well as the study of adsorbed monolayers on liquid surfaces7. Single particle tracking refers to the manual or automatic process of tracing particle trajectories to extract motion information about the transport property of the particle or the physical property of the adjacent medium. Bring the tracer particles into the interface is an effective method to calculate the interfacial viscosity. The motion of tracer particles can be observed by total internal reflection fluorescence microscopy (TIRFM), which is an imaging technique that enables the selective excitation of fluorophores in a thin layer close to the substrate surface inspected[8] (*Fig.A-1, left*). Total internal reflection generates high contrast images with low background fluorescence from unfocused planes since the excitation of fluorophores is limited within a thin layer. Axelrod[9] who found the evanescent wave combined the theory of TIRF with microscope imaging to stimulate fluorescence from matched dyes situated close to the interface and obtain images of the lower cell surface in conjunction with membrane associating dyes at the first time. Since then, the TIRFM has developed rapidly due to its capacity that enables achieve single fluorescent particle tracing by reducing the excitation volume to a thin film at the interface between the sample solution and microscope slide. Because of the best quality of the evanescent wave, the lowest auto fluorescence and scatter and the best signal to background ratio, the prism-type TIRFM (Fig.A-1, right) was selected as the device.

In this study, the interfacial viscosity of liquid/liquid interface was calculated in precise numbers, and the effect by adding surfactants to interface was investigated. Two different dynamic behaviors of single fluorescent nanoparticles existing at the liquid/liquid interface were observed by TIRFM under an external field (orientation movement) and a pure thermal molecular agitation (lateral diffusion), respectively, which is shown in Fig.A-2. The interfacial viscosity can be estimated according to the kinematic velocity of particles. The details of the present work can be divided into following parts:

1 Fluorescence Fe_3O_4/polystyrene nanoparticles (Fe_3O_4/PS Nps) were synthesized by suspension polymerization in water solution. To obtain fluorescent Fe_3O_4/PS Nps, a rhodamine derivative was added during the polymerization. The prepared Fe_3O_4/PS Nps with 30 - 200 nm in size were observed during transmission electron microscopy (TEM) analysis. Dodecane and Fe_3O_4/PS Nps aqueous suspension were added into a particular microcell on a microscope stage and an electromagnet was placed touch to the microcell to create a constant magnetic field. TIRFM with laser light (wavelength 532 nm) was carried out to observe the Fe_3O_4/PS Nps. Fe_3O_4/PS Nps were bearing a constant velocity towards the magnet within a short distance at the interface. The migration of the Fe_3O_4/PS Nps were successfully observed by the TIRFM at the dodecane/water interface. Based on the Stokes viscosity theory and magnetic field force theory, a theoretical formula of the interfacial viscosity was derived with the velocity of Fe_3O_4/PS Nps migration.

In order to obtain the average size of tracer particles, the migrations of the Fe_3O_4/PS Nps in bulk water were recorded first. Whereafter, known size Fe_3O_4/PS Nps were rested at dodecane/water interface. The viscosity of the dodecane/water interface was estimated and value is equal to 1.12 ± 0.04 mPa·s. In addition, the effects of

surfactants, Triton X-100 (TX-100) and sodium dodecylbenzenesulfonate (SDBS), on the interfacial viscosity were investigated. Most notably, a significant decrease of the Fe_3O_4/PS Nps mobility at the interface with surfactants as compared to the corresponding surfactant-free interface was observed due to reduced surface free energy.

2 Polystyrene nanoparticles (PS Nps) were purchased and rested at a series of n-alkane/water interfaces directly. n-alkanes and PS Nps aqueous suspension were added into a particular microcell. Motion trajectories of nano-scaled PS Nps were recorded in real-time and tracked at the interface by TIRFM.

The relation between diffusion coefficient of particle and interfacial viscosity was deduced. From a statistical analysis of particle trajectories, the interfacial viscosities of n-alkane/water were figured out and the results indicated that interfacial viscosity increases with the increasing alkane viscosity. Furthermore, the nature of tracer particles is extremely important in single particle microrheology at interfaces, oversized tracer particle showed an abnormal diffusion due to its change of immersion depth in aqueous phase. The increased concentration of surfactant increases the interfacial viscosity because of the weakened interaction among interfacial molecules. Chain length compatibility of the surfactant and alkane phase has an important effect upon interfacial viscosity. With the same number of carbon atoms of dodecane, sodium dodecyl sulfate (SDS) showed the greatest variation on interfacial viscosity with the increasing concentration among three surfactants (sodium hexadecyl sulfate and sodium octadecyl sulfate). Moreover, at a low concentration, sodium dodecyl benzene sulfonate (SDBS) had a greater influence on the interfacial viscosity than SDS by virtue of its benzene ring. At a high concentration, the factor of compatibility of the alkyl chains occupies the dominant position.

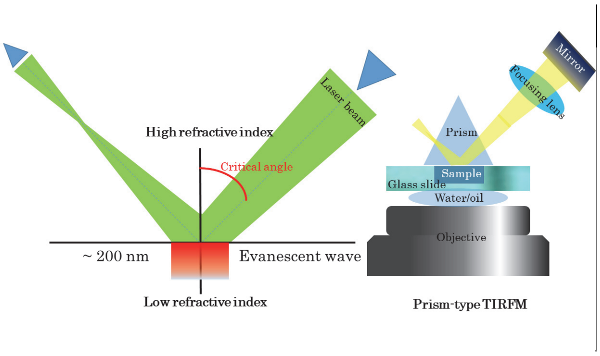

Fig.A-1.A schematic of TIRF and prism-type TIRFM

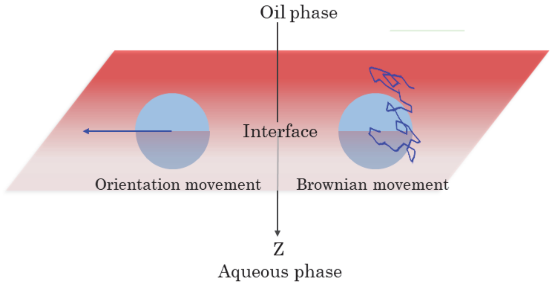

Fig.A-2 A diagram of the two motions

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様式 7

論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

有機溶媒と水溶液の界面（液液界面）化学は，溶媒抽出化学やコロイド化学などの基礎研究から，化粧品や加工食品にかかわる応用研究まで幅広く関係する分野である。液液界面の基礎物性は，上記の分野にとって極めて重要であるが，熱力学的な安定性を意味する界面張力以外の物性はほとんど計測されておらず，特に動的な物性については統一的な物性が存在しない。

そのような研究背景から，本論文では，粘性率という動的な物性に注目して，液液界面の粘性率の測定を行っている。次のような２つの方法を用いて，液液界面の粘性率を評価した。

１）常磁性微粒子である酸化鉄を作製し，それをコアにし，ポリスチレンをシェルにした構造の常磁性ナノ粒子（直径 42 nm）を作製した。これを分散させた水溶液とドデカンを薄層二相マイクロセル内に入れて液液界面を作製した。マイクロセルを倒立顕微鏡のステージ上に置き，全内部反射条件で，液液界面にレーザー光（波長 532 nm）を照射し，マイクロセルの下から対物レンズを用いて，ナノ粒子の泳動を観察した。磁気ナノ粒子を液液界面にトラップさせた後，マイクロセルの外に配置した電磁石を用いて磁場を印加し，磁気ナノ粒子を泳動させた。磁気泳動力と粘性抵抗力が等しいとして，界面における粘性率を求めた。界面活性剤を添加していない系では，得られた粘性率は，ドデカンと水のほぼ中間の値を示した。一方，陰イオン性と非イオン性界面活性剤を添加した結果，いずれの界面活性剤の場合でも，その濃度の増加とともに得られた粘性率の値が増大した。このことは，界面活性剤分子の相互作用により，界面の粘性が増大していることを示している。

２）上記の薄層二相マイクロセルと倒立顕微鏡，全内部反射レーザー励起装置を用い，アルカン／水界面における非磁性の蛍光ナノ微粒子（直径 50 nm，100 nm，200 nm）のブラウン運動を観測した。これらも液液界面にトラップさせることが可能であり，その位置を時間の関数として測定し，平均二乗変位を時間に対してプロットした。その結果，すべての例で，両者の間に比例関係が得られた。そのプロットの傾きは，それら微粒子の液液界面における拡散係数の４倍に相当する。拡散係数と，各微粒子の大きさから，液液界面における粘性率を，アインシュタインーストークスの式から求めた。界面活性剤を添加していない系では，得られた粘性率は，アルカンと水のほぼ中間の値を示した。このことは，アルカン／水界面は，特別な構造を有していないことを示している。一方，様々な長さの炭化水素鎖を有する陰イオン性の界面活性剤を添加した結果，界面活性剤の濃度の増加とともに得られた粘性率の値が増大した。このことは，界面活性剤分子の相互作用により，粘性が増大していることを示している。

以上のように，本論文は，ナノメートルサイズの微粒子を用いて，液液界面の動的物性の１つである粘性率を評価する方法を確立したものである。よって，本論文は博士（理学）の学位論文として十分価値あるものと認める。