Okinawa Institute of Science and Technology Graduate University

BOOK OF ABSTRACTS

Linear and Non-Linear Microrheology of Soft Particle Suspensions

Frank Scheffold

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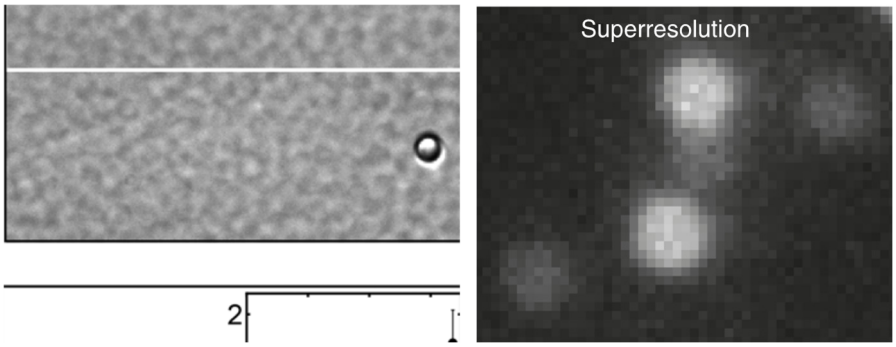
In this talk I will present experimental results on the microscopic dynamics, rheology and microrheology of suspensions consisting of soft colloidal particles. I will discuss the question whether the microscopic motion of particles (individually or averaged as an ensemble) can provide quantitative information about the macroscopic rheological properties in the liquid (glassy) state and in the solid (jammed) state. Weakly attracting particles and reentrant behavior will be covered. I will also investigate the nonlinear intermittent dynamics of probe particles in a colloidal glass. Moreover, I will address the role of friction for soft microgel particles that can weakly interpenetrate. Experimentally, we study micron-sized monodisperse emulsion droplets and microgels. Emulsion droplets are deformable and have a smooth interface (no static friction), but can be made attractive by adding a depletion agent. Microgels are highly deformable and can weakly interpenetrate, which can lead to friction under high compression. To study the motion in dense systems, we use a combination of diffusion wave spectroscopy (DWS), light scattering, optical tweezers and confocal microscopy. To this end, we propsed an improved DWS analysis scheme that accounts for collective scattering contributions and allows us to accurately measure the confined translational motion of densely packed oil droplets. For microgels, we use rheometry, DWS, and two-color super-resolution microscopy with an optical resolution as low as 30nm.



Left: Movement of a 2 mm polystyrene probe particle placed in a nearly index-matched glassy emulsion with a volume fraction of 60 %. The diameter of the probe particle and the emulsion droplets is the same. An optical force is applied to the right and the resulting displacement is shown for a high force [1]. Right: Two-color super-resolution images of highly compressed microgels (eff. volume fraction 190 %) including a pair in contact [2].

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Microrheological Analysis on the Entanglement Properties of Polyelectrolyte Semidilute Solutions

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Entanglements and their effects on the polymer dynamics for polyelectrolyte solutions have been the subject of a long-standing debate over the past 30 years. Many experimental studies reported conflicting information on their viscoelastic properties when compared with those predicted based on the tube model developed for electrically neutral polymers. These existing rheological studies of polyelectrolyte systems with low-viscosity solvents are limited by the accessible frequency range ($\leq$ 100 rad s^{-1}) provided by conventional shear rheometers. To overcome this issue, we employ a diffusing wave spectroscopy (DWS) based microrheological technique to investigate the linear viscoelastic response of aqueous polyelectrolyte solutions containing a high molecular weight poly(sodium styrene suflonate). By measuring the complex modulus over a wide frequency range of $10^1 \leq \omega \leq 10^7$ rad s^{-1} via DWS, we evaluate the polymer concentration effect on the entanglement properties (plateau modulus, reptation time, Rouse time of an entanglement strand) of polyelectrolyte solutions in the semidilute entangled (SE) regime. We show that the power-law dependence of these properties on the polymer concentration, obtained using DWS, agrees well with the predicted dependence for salt-free polyelectrolyte solutions in the SE regime. Thus, our results demonstrate that the tube concept developed for neutral polymers can be applicable to polyelectrolyte systems.

Passive Microrheology in DNA-Hydrogels

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Characterization of new types of hydrogels made of DNA-nanostars, proteins or other biopolymers is increasingly relevant for the development of bio-sensors and diagnostic tools. Their high cost in the development stage makes microrheology an excellent tool for testing their vicoelastic properties as function of temperature and many physical and chemical parameters in very small volumes. I will present and compare microrheology in DNA-hydrogels, based on measuring the thermal fluctuations of probe colloids either trapped by optical tweezers [1], or from scattering methods such as Dynamic Light Scattering or Diffuse Wave Spectroscopy [2-4]. These methods allow us to investigate the systems in the frequency range of 1 - 10^5 Hz. In particular the high frequencies can give us information on the local persistence length of the biopolymers and characteristic relaxation times as function of solvent quality. I will discuss technical aspects and comparison with bulk-rheology.

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Construction of Three-Dimensional Structures of Cells using Combinations of Micro/Nano Process, Computational Origami and Cell Origami Techniques

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Recently, a field called "computational origami", which is based on "folding" and calculates geometric problems necessary to create a three-dimensional (3D) shape, has made great progress. In this study, by implementing this computational origami technique at the cellular level, we establish a method for efficiently forming targeted micro-sized structures by folding cells cultured in a two-dimensional plane like origami. Furthermore, we aim to assemble the small solid like a block with the size of several cell and construct a larger 3D structure.

We also develop a micro/nano-sized pattern substrate in which cancer cells spontaneously construct three-dimensional tumors. Nano-sized surface roughness is created inside the micro-sized pattern. In this research, we are able to form the tumors that are closer to the one in the living body *in vitro*. It is possible to observe living tumor in real time, which was not possible until now. Furthermore, as a result of seeding cells with differences in malignancy on the substrate, we found that their behaviors were different. I now try to set up a company to apply the development of new cancer drugs using the substrate.

Key Words: Cell origami, Computational Origami, Regeneration medicine, 3D culture of cancer cells, Bio-MEMS

Rheo-XPCS Studies of Yielding and Recovery in Nanocolloidal Soft Glasses

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Instabilities and Flow-Induced Structures in Nematic Liquid Crystals

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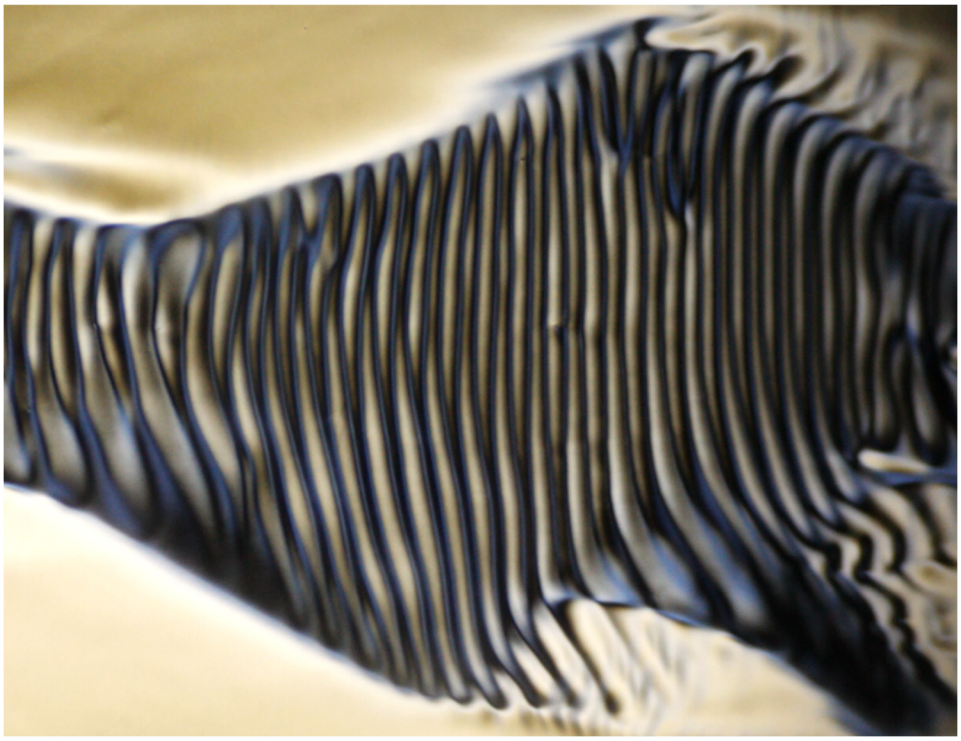
Lyotropic chromonic liquid crystal (LCLC) solutions in the nematic phase have peculiar properties. They are tumbling materials, which means that flows can easily destabilize the director alignment, and they possess a large elastic anisotropy where twist elastic deformations are energetically much cheaper than splay or bend deformations. We show how these characteristics can be exploited to induce controlled growth morphology transitions from the generic dense-branching growth to dendritic growth in the viscous-fingering instability, and how they lead to unique structure formation as the LCLC solutions are driven out-of-equilibrium by a pressure-driven flow in a microfluidic channel. In particular, we report the surprising emergence of chiral domains in the material despite the achiral nature of the material. The chirality results from a periodic double-twist deformation of the liquid crystal and leads to striking stripe patterns vertical to the flow direction. We discuss the mechanism of this unique pathway to spontaneous mirror symmetry breaking and rationalize the selection of a well-defined period of the chiral domains.



Multiple Length Scale Flow and Mechanics of Complex Bacterial Nanocellulose Structures

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Bacterial cellulose biofilms are complex networks of strong interwoven nanofibers that control transport and protect bacterial colonies in the film. Design of diverse applications of these bacterial cellulose films also relies on understanding and controlling transport through the fiber mesh and its micromechanical and microrheological response. Bacterial nanocellulose fibers have a surprising range of characteristic length scales, resulting in the formation of complex mesh structures with the potential for significant variations in permeability and flow over microfluidic and smaller dimensions. This talk will examine the transport, deformation, and flow behavior that can be experienced in native bacterial cellulose structures and present multiple examples of systems that have been engineered and processed to expand and practically exploit these properties.

Flow and Alignment of Protein Nanofibril Suspensions

Tatiana Porto dos Santos

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Protein nanofibrils (PNFs) obtained through the self-assembly of milk whey proteins or peptides have gained increased attention in food and material science-related applications since they serve as good rheological and texture modifiers, as well as gelling and stabilizing agents. Although the physical and technological properties of such a prominent colloidal material have been recently investigated (e.g., effect of physico-chemical conditions, stabilization of emulsions, formation of gels, etc.), the flow-induced modification of the PNFs in the isotropic state has not been systematically explored yet. In this work, a simple shear-dominated microfluidic platform is employed to assess the effect of flow (i.e., onset and pattern of alignment and velocity profile) on β-lactoglobulin PNFs with different flexibilities. In particular, we used a combination of flow-induced birefringence (FIB), μ-particle image velocimetry (μ-PIV), and conventional rheological characterization techniques, to understand the onset of flow-induced alignment of β-lactoglobulin PNFs. A universal curve involving the Péclet number (*Pe*, a dimensionless number quantifying the relative strength between the shear rate and the rotational diffusion coefficient of the nanofibrils) and an alignment parameter (birefringence signal Δn normalized by the mass fraction of protein φ), could be established from our systematic experimental results to predict the alignment and flow profile of PNFs under shear-dominated flow (in the range of $1 < Pe < 10^5$ and $10^{-4} < \Delta n/\varphi < 3\times10^{-3}$). Our results shed insights on the physical-mechanical fate of PNFs under various manufacturing operations, e.g., processes of PNFs in the scale-up food industry as well as when passing through gastrointestinal digestive systems.

Droplets and Microfluidics: What's Next?

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As droplet and microfluidic technologies have found increasing applications in a variety of areas, such as biotechnology and chemical analysis, the advantages and limitations of existing approaches have been well defined. To take droplets and microfluidics to new capacities, we need to think beyond conventional boundaries in the fluid systems, device fabrication and other operational aspects. In this talk, I will share some of our efforts to go beyond water-oil based droplets and beyond conventional PDMS microfluidic devices. While these different systems, such as all-aqueous droplets and stimuli-responsive microfluidic devices, may require new ways to operate and manipulate, they also potentially open up new applications that require more dynamic assembly of macromolecules and inspire new ways to enhance yields of reactions. These examples will hopefully inspire a stimulating discussion with the audience on the next stage of developments of droplet and microfluidics.

Cortical Flow Beneath the Plasma Membrane Induces Cluster Cell Migration

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Cellular migration is a key component of numerous biological processes, including the morphogenesis of multicellular organisms, wound healing, and cancer metastasis, where cells adhere to each other to form a cluster and collectively migrate. Although the mechanisms controlling single-cell migration are relatively well understood, those underlying multiple-cell migration still remain unclear. In cell migration it is known that the flow of actomyosin networks beneath the plasma membrane, called cortical flow, plays an important role, but the relations between cell migration and cortical flow are still unclear. In this presentation, we provide some mechanical model that describes the dynamics of cell membrane and satisfies force balance on the cell membrane, and demonstrate that the combination between cortical flow and friction with the surrounding medium induces not only single cell but also multiple (cluster) cell migration.

Impact of Peplomer Charge Heterogeneity on the Rotational Diffusivity of a Suspension of Coronavirions

Eliot Fried

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Diffusing Wave Spectroscopy of Colloidal Dispersions

Eric Furst

Department of Chemical and Biomolecular Engineering, University of Delaware, Delaware, USA

A Unified Framework to Describe Shear- and Extension-Induced Alignment of Macromolecules of Various Flexibility

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Macromolecules, such as polymer chains in solution and colloidal rods in dispersion, can adopt a favourable orientation under flow. However, how the flow type (e.g., shear and extensional deformations) affects the alignment of macromolecules remains unclear, especially for semi-flexible macromolecules. We tackle this problem by analysing the shear- and extension-induced alignment for a library of macromolecules with distinct flexibilities, spanning from rigid colloidal rods to more flexible polyelectrolytes in solution. We combine microfluidics with flow-induced birefringence to probe and compare the macromolecular alignment under steady extensional and shear deformations. We describe a generalized relationship for the effectiveness of extensional flows, compared to shearing flow, at inducing the alignment of macromolecules of various flexibility. This quantitative understanding could potentially serve as a methodology to retrieve a statistically robust measure of the macromolecule flexibility that is model-free.

Exploring Complex Rheological Behavior of Soft Materials with Large Amplitude Oscillatory Shear (LAOS)

Jun Dong Park

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A lot of soft materials, such as colloidal suspensions and emulsions, demonstrates complex rheological behavior that is originated from their characteristic microstructure and dynamics. On better understand the complicated rheological behaviors of soft materials, rheologists have applied numerous types of deformation to them and examined their responses. Among the various rheological test protocols, Large Amplitude Oscillatory Shear (LAOS), under which materials experience wide range of rheological and physical transition such as recovery and yielding, has served as an important tool to explore complex rheological behavior. Especially, the advent of the Sequence of Physical Processes (SPP) technique that can provide temporally resolved information on the rheological transition during oscillatory shear has expanded the potential of LAOS test.

In this presentation, two interesting cases where LAOS-SPP analysis effectively solve complex problems on the soft materials will be introduced. As a first example, the nonlinear rheological behavior analysis of lithium-ion battery anode slurry will be presented. Lithium-ion battery anode slurry consists of three different components of graphite (active material), carboxy-methyl cellulose (binder), and carbon black (conductive material). The complex composition leads to complex rheological behavior such as two-step yielding in dynamic strain or stress sweep tests, whose physical origin has never been clearly understood. By investigating the intra-cycle rheological transition via LAOS-SPP analysis, we reveal that the two-step yielding of anode slurry is attributed to different physical origins. It is shown that the first yielding step is linked to attractive interaction between graphite particles and resulted network structure. On the other hand, the second yielding step is attributed to the interaction between carboxy-methyl cellulose on the graphite surface.

Another example is development of sensory attribute predictive model for cosmetic formulations with LAOS. In cosmetics industry, there have been many attempts to establish predictive model for sensory attribute based on rheological parameters as it can replace expensive and laborious panel test. Inspired by an analogy between the application process of cosmetics and LAOS, we suggest a novel predictive model for the spreadability of cosmetic formulations with the LAOS-SPP analysis parameters and machine learning techniques. Our results indicate the importance of the LAOS analysis in texture perception mechanism of cosmetics, and how LAOS analysis can be combined with machine learning techniques to solve a practical problem.

Optimized Microfluidic Device for Homogeneous Uniaxial and Biaxial Elongation of Mobile Fluids

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The Optimized Shape Cross-slot Extensional Rheometer (OSCER) is a stagnation point microfluidic device, based on the planar cross-slot geometry, which has a numerically-optimized wall profile producing near ideal planar elongation [1,2]. In this work, a similar shape optimization strategy is employed on a '6-arm cross-slot' [3,4], resulting in a three-dimensional (3D) geometry that produces almost ideal uniaxial (and biaxial) elongation. The optimal geometry is fabricated to high precision in fused silica glass using the subtractive 3D-printing technique of selective laser-induced etching. Micro-particle image velocimetry (μ-PIV), for flow of a Newtonian glycerol/water mixed solvent that is refractive index-matched to the glass microdevice, demonstrates that the device does indeed produce the velocity profiles expected for both uniaxial and biaxial extension (depending on the direction of the imposed flow). The new device (called the Optimized Uni and Biaxial Extensional Rheometer, OUBER) is employed, along with the pre-existing OSCER device, to compare between the uniaxial, planar and biaxial extensional flow response of a range of dilute poly(acrylamide) solutions. For this, μ-PIV and pressure loss measurements are combined, revealing significant differences between the viscoelastic response (i.e., flow field perturbation, stability conditions, and apparent extensional viscosity) in each mode of extension, that may not be well predicted by current theories.

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Microheterogeneities Everywhere: A Microrheological Approach for Visualization and Characterization

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Microheterogeneities, i.e. heterogeneities on an intermediate length scale of 0.1 – 10 μm are ubiquitous in nature and technology. They occur in concentrated colloidal suspensions widely used in all kinds of coatings and inks, in industrial thickening agents, but also in biopolymer solutions or gels used in tissue engineering and 3D bioprinting. Often, such heterogeneities are hardly accessible to conventional imaging techniques due to low contrast. Here we present a new approach for analysis of multi particle tracking (MPT) microrheology data, the so-called Micro-Rheo-Mapping technique, which relies on a "rheological contrast" for imaging. The method takes advantage of the large amount of short particle trajectories usually discarded in the analysis of MPT data. All recorded trajectories are used to create overlay images of subsequent frames recorded in a movie and in combination with Voronoi triangulation based on long MSDs typically evaluated in MPT experiments an accurate characterization of sample heterogeneities is enabled.

In concentrated colloidal dispersions dilute fluid regions coexist with denser crystalline or gel-like regions. The fraction of these phases, particle concentration in each phase as well as the size and modulus of the micro-crystals or gel-like aggregates could be obtained using MPT and bulk rheology varying with particle interaction was correlated to these microstructural features. Above the colloidal glass transition strong heterogeneities and different local particle mobility in repulsive and attractive arrested states was found.

The high thickening efficiency of commercial Carbopol™ thickeners is a consequence of the heterogeneous microstructure with regions of different crosslink densities and the bulk shear modulus strongly depends on the fraction of highly crosslinked areas inaccessible for MPT tracers. The various Carbopol types differ in terms of the microgel aggregate size and the thickness of the shell of dangling polymer chains surrounding them.

The network structure of lyophilized collagen I (Coll) fibers distributed in hydrochloric acid solutions was directly visualized using MPT. Freely diffusing tracer particles yielded viscosities indicating that, irrespective of concentration, a constant amount of Coll is dissolved in the aqueous phase. Particles found elastically trapped within fibrous Coll structures revealed a structure comprising multiple fiber bundles with dense regions in-accessible to tracers and elastic regions of different stiffness in between. Bulky aggregates exist even at low Coll concentrations, and a network of slender fibers evolves above the sol–gel transition.

Based on MPT investigations a generic design concept for tuning the elasticity of polymer and particle gels was developed. Targeted micro-phase separation resulted in gel elasticity variations of more than two orders of magnitude. Structural heterogeneity was enhanced in alginate hydrogels by accelerating crosslinking kinetics, generating higher elastic materials. Adding PVA prevented alginate crosslinking, producing softer, more homogeneous hydrogels. Introducing PVA into gelatin gels resulted in more heterogeneous materials with higher elasticity due to micro-phase separation. Viscous inclusions were formed leading to pronounced slip behavior and superior printing quality of complex 3D constructs as well as high HepG2 and NHDF cell viability due to reduced shear damage during extrusion. Higher ionic strength in Laponite-based hydrogels induced nanoparticle aggregation, producing materials of higher elasticity. The formation of polymer/nanoclay clusters led to rapid brittle failure, facilitating extrusion and therefore enhancing printing quality as well as cell viability. Targeted introduction of micro-heterogeneities in bioinks through micro-phase separation is an effective technique for high resolution 3D printing of complex constructs with high cell viability. The size of the heterogeneities, however, has to be substantially smaller than the targeted feature size in order to achieve good printing quality.

Development of the Ultrafast Camera for Single-Molecule Imaging and the Discovery of Metastable Nano-Liquid Signaling Platforms on the Cell Membrane

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The spatial resolution of fluorescence microscopy has recently been greatly enhanced. However, improvements in temporal resolution have been limited, despite their importance for examining living cells. In the first part of my presentation, I will talk about an ultrafast camera system we developed that enables the highest time resolutions in single fluorescent-molecule imaging to date, along with theoretical frameworks for the analysis of single-molecule trajectories in the plasma membrane. We showed that the temporal resolution is photon-limited by fluorophore photophysics: 33 (100) μs with a single-molecule localization precision of 34 (20) nm for Cy3, the optimal fluorophore we found. This camera successfully detected fast hop diffusion of membrane molecules in the plasma membrane (PM), detectable previously only in the apical PM by using less preferable 40-nm gold probes, thus helping to elucidate the principles governing the PM organization. Furthermore, this camera allows simultaneous data acquisitions for PALM/dSTORM at as fast as 1 kHz, with 29/19 nm localization precisions in the 640×640 pixel view-field.

In the second part of my presentation, I will address the crosstalk of cellular signaling pathways essential for coordinated cell responses. We suspected the existence of scaffolding platforms that specifically undertake signal crosstalk and integration. Using advanced single-molecule imaging, we found a nanometer-scale liquid-like platform for integrating the signals downstream from GPI-anchored receptors and receptor-type tyrosine kinases. The platform employs some of the focal adhesion proteins, including integrin, talin, RIAM, VASP, and zyxin, but is distinct from focal adhesions, and is thus termed iTRVZ. The iTRVZ formation is driven by the protein liquid-liquid phase separation and the interactions with the raft domains in the plasma membrane and cortical actin. iTRVZ non-linearly integrates the two distinctly different receptor signals, and thus works as an AND gate and noise filter.

Crack Tip Analysis for Fast Moving Crack in Elastomers

Kenji Urayama

Department of Material Chemistry, Kyoto University, Kyoto, Japan

Understanding of crack propagation in soft materials such as elastomers and gels remains a challenging issue. Large deformability and finite viscoelasticity, both of which are absent in hard materials such as metal and ceramic, complicate the crack growth phenomena of elastomers. We employ the Mode-I geometry (**Fig. 1**) to analyze the crack propagation in stretched elastomers. A wide-width rubber specimen is stretched to a target elongation of λ, and subsequently, a crack is initiated by a scissors from the side of the specimen. The resulting crack propagation is observed by a high-speed camera combined with digital image correlation (DIC) technique. This experiment enables to characterize the details of a propagating crack including the steady-state velocity, the area of finite strain caused by the crack, the crack-tip opening displacement, and the strain singularity near the crack-tip.

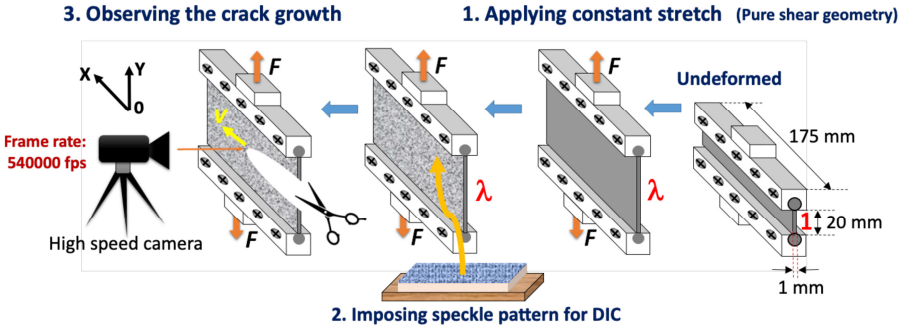

Fig. 1 Experimental setup for the observation of the Mode-I crack growth in elastomers.

In this talk, I introduce our recent studies using this technique [1], i.e., an extremely high-speed crack exceeding the ultrasound speed of the elastomers [2], a crack propagating through the elastomers undergoing strain-induced crystallization [3], a crack in the elastomers with anisotropic damage [4], and a stationary crack under various types of biaxial strain [5].

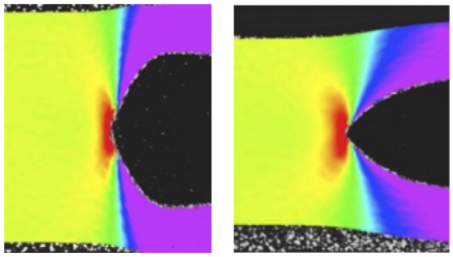

Fig. 2 Strain distribution near the crack-tip of the fast moving cracks of about 10 m/s in the styrene butadiene rubbers with the filler contents of (left) 5 and (right) 21 vol%.

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Confinement and Complex Viscosity

Jeff Giacomin

Chemical Engineering Department, Polymers Research Group, Queens University, Kingston, Ontario, Canada

Whereas much is known about the complex viscosity of polymeric liquids, far less is understood about the behaviour of this material function when macromolecules are confined. By confined, we mean that the gap along the velocity gradient is small enough to reorient the polymers. We examine classical analytical solutions [Park and Fuller, JNNFM, 18, 111 (1985)] for a confined rigid dumbbell suspension in small-amplitude oscillatory shear flow. We test these analytical solutions against the measured effects of confinement on both parts of the complex viscosity of a carbopol suspension and three polystyrene solutions.

LIST OF POSTER PRESENTATIONS

1. Arisa Yokokoji, OIST, Japan:
   Rheological Effects on the Cross-Slot Flow Instability
2. Mauricio Andres Rios Maciel, OIST, Japan:
   Duplex Electrochemical Microfluidic Biosensor for COVID-19 Antibody Detection
3. Atsushi Matsumoto, University of Fukui, Japan:
   Counterion Condensation of Poly(ionic liquid)s in Solvents with Varying Dielectric Constants
4. Jiangming Wu, OIST, Japan:
   Diffusion and Alignment of Colloidal Rods in Highly Viscous Newtonian Solvents
5. Fabian Hillebrand, OIST, Japan:
   Flow of a Wormlike Micellar Solution Over a Long Cavity
6. Mohamed Abdelgawad, OIST, Japan:
   Probing the Extensional Properties of Yield Stress Fluids
7. Erika Fukuhara, OIST, Japan:
   Analysis of *Bacillus subtilis* Malate Dehydrogenase and Citrate Synthase Metabolon Formation in Phase Separated Protein Droplets
8. Yusaku Abe, Waseda University, Japan:
   Modeling and Manipulation of Self-Assembly Process Toward Controlling Functional Molecular Pattern Formation
9. Stelios Varchanis, OIST, Japan:
   Fringe Instability Downstream of a Sphere Sedimenting in a Viscoelastic Fluid
10. Daniel Carlson, OIST, Japan:
    Volumetric Evolution of Elastic Turbulence in Porous Media
11. Simon Haward, OIST, Japan:
    Design and Fabrication of an Optimized "6-Arm Cross-Slot" Device
12. Cameron Hopkins, OIST, Japan:
    Upstream Wall Vortices in Viscoelastic Flow Past a Cylinder

13. Vincenzo Calabrese, OIST, Japan:
    Block Copolymer Worms under Flow: Shear and Stretch
14. Stoffel Jansens, OIST, Japan:
    Effect of a Surface Tension Imbalance on a Partly Submerged Cylinder
15. Jose Muneton Diaz, University of Fribourg, Switzerland:
    From Interparticle Potentials to Rheological Properties of Microgels
16. Alessandro Bevilacqua, OIST, Japan:
    Shaping Enzyme Activity by Tuning Protocells' Size and Composition
17. San To Chan, OIST, Japan:
    Prevention of Edge Fracture using a Liquid Metal Sealant