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Current Opinion in
Colloid & Interface Science

Complex fluid product microstructure imaging with light sheet fluorescence microscopy

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Light sheet fluorescence microscopy (LSFM), uses a thin sheet laser illumination to rapidly image samples with greatly reduced photodamage versus previous techniques. The flexibility of the technique has powered significant advances in biology research by connecting length scales from individual cells to entire developmental embryos. For similar reasons, LSFM is poised to enhance mesoscale imaging of complex fluid microstructures in fundamental and commercial systems. This work introduces the LSFM technique and reviews some of its complex fluid applications along with examples of its use in food, biomaterial, and emulsion product samples. The technique is shown to nicely complement existing complex fluid characterization methods because of its breadth of length scale resolution and its lack of photobleaching effects. New product applications for LSFM are identified and discussed, suggesting additional integration with existing techniques.

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Current Opinion in Colloid & Interface Science 2025, **77**:101916

This review comes from a themed issue on **Cosmetics**

Edited by **Samiul Amin** and **Gerardo Palazzo**

For a complete overview see the Issue and the Editorial

https://doi.org/10.1016/j.cocis.2025.101916

Introduction

The microstructure of commercial complex fluid products is essential to the successful development of stability, multifunctional performance, and value for consumers. The reason is the critical contributions made to flow and texture that impact perception, feel, delivery, and manufacture of these materials.

One of the most compelling values of complex fluid formulations for commercial product development is the ability of small amounts of ingredients, usually in water, to alter the bulk flow and performance properties of fluids so that a significant margin can be charged to a consumer. Adding even less than one percent of a polymer, attractive colloid, or surfactant to water, for example, can transform a low-viscosity liquid into a viscous fluid that exhibits much more complex flow behavior. The reason for such unique performance is the formation of mesostructures, like micelles in surfactants and gels in polymer or colloidal systems, that resist flow in complex ways and produce combinations of properties that are commercially useful.

An example is shear-thinning behavior, where a product flows slowly with high viscosity at small shear rates but thins significantly when strongly sheared, like shampoo [1]. Because of the strong structure-flow coupling, understanding the microstructure and mesostructure of formulated products is essential to their design, use, and troubleshooting, but so too is the ability to connect these smaller-scale structures to larger-scale properties and behaviors. Both goals represent significant grand challenges in fundamental and applied complex fluid research [2].

The design of complex fluid products requires an understanding not only of their desired flow properties, attributes like shear-thinning behavior [3] or yield stress [4], but how the fluid structure can best deliver these properties. A critical aspect of complex fluid product formulations is their memory of processing history. Microstructure can be uniquely encoded into a product, differing from other products with identical composition, simply because of a different cumulative set of flow histories [1,5].

As consumers demand more and requirements change with the evolution of legislation and standards, more rapid understanding of complex fluid microstructures is essential, especially with respect to the dynamics as the structures age, evolve, and are used. Significant progress has been made in this space using techniques like confocal laser scanning microscopy, CLSM, for micron-scale insights, and small-angle x-ray scattering, SAXS, for smaller-scale study. A new imaging technique that is used extensively by biological scientists, as well as physicists studying granular materials, is light sheet

fluorescence microscopy, LSFM. LSFM offers a unique opportunity to develop structure–function relationships over several orders of magnitude in length scale, from micron to millimeter scales, providing a powerful complement to foundational methods like CLSM and SAXS.

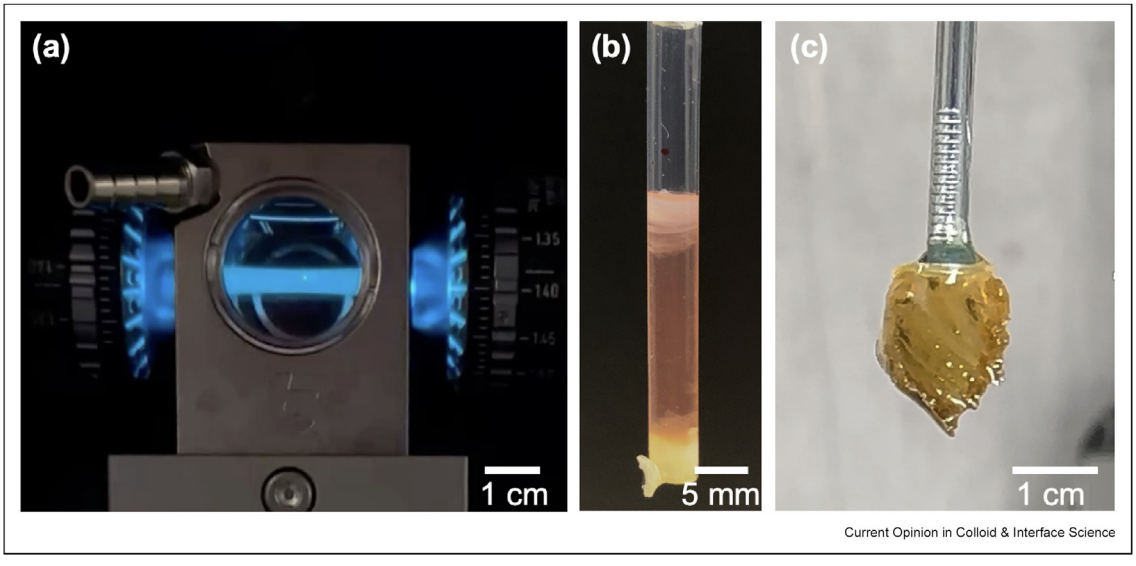
Here we argue that LSFM has significant potential to expand capability in the design, development, and production of complex fluid formulations by companies spanning the cosmetic, food, cleaning, and pharmaceutical industries. All of these industries have products that deliver benefits perceived on the millimeter and larger length scales, but do so through the complex self-assembly, flow, and aging of much smaller micron-scale and mesoscale structures. LSFM offers a unique ability to perform mesoscale imaging as a complement to smaller-scale structural probes. Commercial complex fluid formulations are often a rich source of fundamental problems [1], so we see the expansion of LSFM techniques into more complex fluid labs as beneficial to fundamental researchers in industry and academia. We first introduce the LSFM technique and its amazing utilization in biological fields, then draw a parallel with confocal imaging approaches to the study of complex fluids to illustrate how LSFM can expand what is now possible. We then use diverse examples of complex fluid microstructures and their larger-scale forms, imaged by LSFM, to motivate our commercial and academic colleagues to explore this exciting technique in the formulated product field.

Mesoscale imaging by light sheet fluorescence microscopy

Light sheet fluorescence microscopy, LSFM, also known as selective plane illumination microscopy, SPIM, has had a tremendous impact on biological studies in the last two decades [6–8]. A key difference from traditional microscopy is the use of an imaging objective that is at 90° to the illumination plane, eliminating the out-of-focus fluorescence present in confocal light paths, Figure 1a. This difference enables fast imaging of significant sample sizes but with significantly reduced phototoxicity or photobleaching because only a thin plane of the sample is illuminated, while providing similar optical section sizes to confocal methods [9]. Three-dimensional light sheet imaging of samples requires an additional rotation of either the sample, Figure 1b, or the illumination system, enhancing spatial and temporal coverage. The ability to perform relatively fast imaging of millimeter-scale samples has had a significant impact on developmental biology studies as it enables researchers to follow cell movement and differentiation in entire embryos [10,11]. It is this same versatility for imaging microscopic and macroscopic length scales in space and time that makes the technique so exciting for the study of the synthetic and formulated analogs of biological matter: complex fluids and their associated products.

As with any technique, there are some compromises to be made for sample selection and preparation as well as imaging. While LSFM can image millimeter-scale

Figure 1



(**a**) Experimental geometry of light sheet generated by two opposing illumination microscope objectives on a Zeiss Lightsheet Z.1. The third emission objective is visible through the sample cell window behind the cell at a right angle to the light sheet. (**b**) The closed-end PTFE tube (OD = 5 mm) with refractive index of 1.33 can be used as a sample holder for light sheet imaging of a water-based fluid. (**c**) A meat sample cleared of most of its lipids attached to a nail head sample holder for imaging.

samples, scattering by concentrated systems must be eliminated by "clearing" the samples, Figure 1c, to render them transparent so only labeled components are imaged [12]. Because cosmetic and consumer products are formulated with lipid-based materials like surfactants, waxes, and oils, the method of LSFM is especially suited to the study of the structure and its time evolution. Clearing methods developed for embryos and other biological samples can be adapted easily or directly reapplied to image the most relevant structures and their dynamics, with the obvious caveat that clearing procedures could potentially alter the structure or processes under study. However, an interesting parallel between fundamental biological imaging studies and designs of commercial complex fluids is that many products are formulated to be transparent by matching refractive indices, in effect preprocessing product samples for advanced imaging study. Products that are already transparent can be imaged and studied for structural variation and evolution with only the addition of fluorescent tracer molecules or particles to track structural elements [13,14].

A recent review highlighted the importance of the LSFM method for soft matter research [15], arguing LSFM is poised for a parallel path to that of confocal microscopy transitioning from a biological imaging tool to one that has made significant contributions to fundamental and applied complex fluid work on colloidal [16,17] and polymeric [18,19] systems. The review also highlighted the extensive history of LSFM technique usage to study macroscopic particle structures, packing, and flow by granular material researchers [15].

Product application areas

Commercially formulated products derive their value from their microstructural elements that perform functions like rheology modification, stabilization, and delivery of active ingredients. LSFM offers a way to image these elements and structures by selective tagging and then to follow the evolution of the spatial position and distribution with time, processing, and compositional fluctuations. Below are several specific examples to demonstrate the diverse range of samples and structures that can be studied, drawing on structural elements and attributes common to foods, cosmetics, and other consumer products.

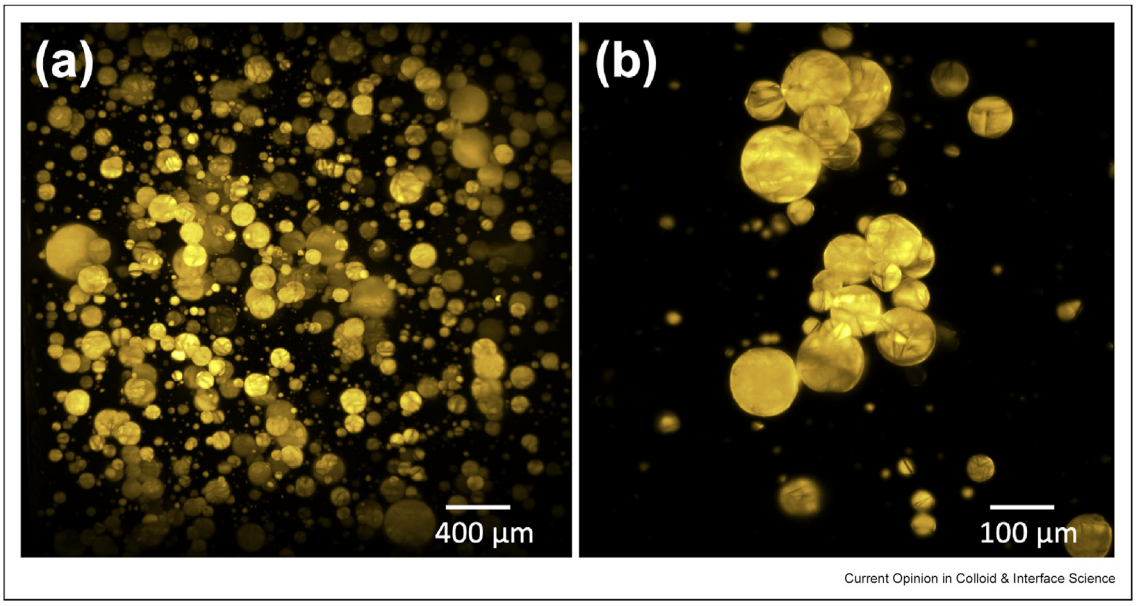
Arrested emulsions

Emulsion droplets are a core component of many food, cosmetic, and consumer products. As a result, the state of droplets at various stages of formulation, usage, and shelf life is crucial for ensuring the emulsion has the desired function for a given application. This involves mapping information at different length scales, including internally partitioned crystals [20], surface-adsorbed colloids [21], and individual droplets that create a product with defined viscosity and elasticity. Generally, emulsions are not thermodynamically stable, as the droplets tend to coalesce to reduce the surface energy, but formulated products often include stabilizers like surfactants or polymers intended to prevent aggregation, coalescence, and creaming instabilities. Understanding the actual state realized in an emulsion is therefore a critical metric of quality, performance, and stability.

Although droplet coalescence is an undesired phenomenon in emulsions, the formation of an arrested structure is essential in some food products, such as whipped cream and ice cream, where the arrested fat droplets build a 3D network in the continuous phase, leading to the stabilization of the product. Mapping and understanding the arrested network at the elastic network scale, the droplet scale, and the larger emulsion scale provide valuable insights that can be used during product formulation. LSFM can provide visualization and quantification all of the information at these length scales in an emulsion. Along with bulk rheology data, LSFM can map the memory and history encoded within these structures and explain how and why a system flows as it does. Figure 2a shows a paraffin-hexadecane emulsion, allowing evaluation of the size distribution of droplets over several orders of magnitude. Also visible is the level of stability between droplets, as many are dispersed individually, but some display partial or arrested coalescence. If the droplets have elasticity due to an internal [22] or surface [21] aggregated structure, that elasticity resists coalescence and can balance interfacial tension to arrest the coalescence process at an intermediate state, forming various metastable shapes [23,24]. Figure 2b shows a close-up of clusters of droplets that display arrested coalescence behavior, including a liquid neck bridging them and the internal wax crystals that appear dark and provide the aggregated elastic structure responsible for the arrest. Figure 2 highlights the strength of the LSFM technique for imaging complex fluid microstructures over a broad range of length scales from the level at which consumers interact with, and perceive, the product performance down to the scale of individual micron-scale droplets and their submicron microstructural elements. Great insights into structure—function relationships for colloidal gel structures have been realized by CLSM mapping of the elements necessary for fluid elasticity [25], and the same sort of understanding is needed for emulsions like those in Figure 2 with capillarity—driven interactions that affect fluid rheology in unique ways.

Beyond the holistic analysis of microstructural elements and their bulk forms, LSFM also enables rapid and highly specific measurement of details of individual dispersed phase elements. For example, recent work showed the feasibility of DNA analysis of individual

Figure 2



(**a**) Light sheet fluorescence microscopy (LSFM), 3D reconstruction of droplets of hexadecane-containing paraffin wax crystals imaged within a full emulsion environment. (**b**) Zoomed-in view of specific droplets with stable and arrested structures. LSFM enables resolution of small details within the droplets, like the arrested neck structure between droplets. Oil is stained here with Nile Red for fluorescence of the liquid phase, while the submicron-thick wax platelet crystals inside appear dark. .

droplets in an emulsion using LSFM [26]. Commercial applications of that level of rapid, specific analysis of a product could be advantageous to testing for active ingredient viability, efficiency of mixing processes, and safety.

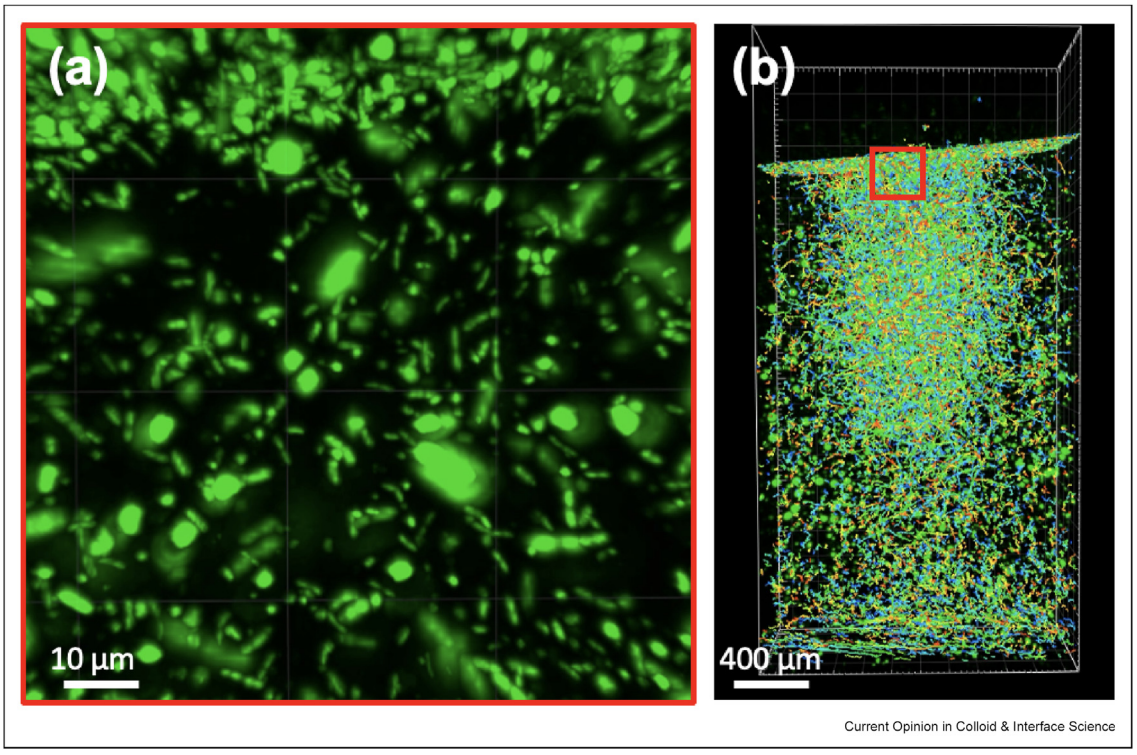
Bacterial tracking in *A. xylinum* culture

Bacterial growth of biofilms can be a dangerous phenomenon, plaguing medical and manufacturing settings but also endangering personal care products and their consumers if not controlled. A recent study [27] followed the growth of a polysaccharide biofilm by *V. cholerae* bacteria using LSFM, mapping their local levels of mobility, entrapment, and fate to understand how expansion is made possible and stabilized. Many biofilms, however, actually yield useful components for consumer and food products like polysaccharide rheology modifiers and hydrogels. An example is the growth of bacterial cellulose, which occurs at the air-liquid interface of an aqueous culture of yeast and *Acetobacter xylinum* bacteria. Food products like Balsamic vinegar, Kombucha, and Nata de coco are made when these organisms ferment sugar solutions to produce acetic acid and pure cellulose fiber mesh structures. The application of bacterial nanocellulose fibers is an incredibly active area of research spanning food, biomedical, pharmaceutical, and advanced materials [28], so more detailed spatiotemporal resolution of their structure forming processes would provide direct design and development guidance.

A bacterial cellulose system offers an excellent use of LSFM to not only capture the live cell behavior in static conditions but also the detailed cell communication and interaction during the dynamic formation of large millimeter-scale structures. How these bacteria grow, reproduce, move, manufacture cellulose, and build a well-organized layered structure has been a research question for a long time, which can be addressed by detailed live bacterial imaging during the process using LSFM. Labeling the live cells with a stain to enable tracking of motion and health in the entire culture provides a dynamic snapshot of a multiscale structure useful for numerous types of cosmetic and consumer products.

Figure 3a shows a close-up view of the green-labeled live bacteria (small, rodlike) and yeast (larger ellipses) within a closed-end polytetrafluoroethylene (PTFE) (refractive index to water, $\sim$ 1.33) tube (OD = 5 mm). The level of detail shown is typical, enabling tracking of all visible cells in the total sample volume over a distance 40 times greater than the field of view in Figure 3a. Figure 3b shows a 3D rendering of all microbe trajectories mapped during the first 30 min of growth. Since the incubation of these live systems can last over

Figure 3



(**a**) Zoomed-in view of green-labeled yeast (large) and bacteria (smaller, rodlike) within a cellulose pellicle biofilm. (**b**) Zoomed-out view of sample holding the bacterial culture showing trajectories of all the visible bacteria over the first 10 min of the experiment.

14 days, the same sample volume can be assessed over a long period of time in an automated way without photobleaching it, mapping the full culture's behavior. Practical issues require some maintenance, like the reduction in fluorescence intensity due to bacterial reproduction, which can be addressed by additional fluorescent dye injection inside the sample cell, minimizing disturbance of the culture.

Assuming the tracks in Figure 3b reflect the production of cellulose fibers and their orientations, the overall structure of the entire pellicle biofilm is mapped as it develops, evolves, and equilibrates in time and space. The LSFM technique offers a powerful ability to move from cellular dimensions to an entire experimental volume, linking the microstructural and mesostructural data needed to understand a complex fluid medium and the product and processes they will encounter or be used within.

In addition to microbial communities [29], LSFM has also been used for live cell imaging of single mammalian and plant cells to map their pattern behavior responses to trigger stimuli [30]. However, the benefit of this method is not limited to biological samples; it can also be beneficial for studying short-term and long-term behavior of consumer, food, and cosmetic products in one comprehensive experiment, whether in static behavior or dynamic mode. Foods, cosmetics, and consumer products are increasingly complex, often incorporating biological components and even active bacterial or enzymatic ingredients. Examples include probiotic foods [31], laundry and nutritional enzymes, and agricultural biostimulants [32]. The complex interactions of product formulation stability, flow, and protection of bacterial viability in these systems can all be assessed using LSFM imaging.

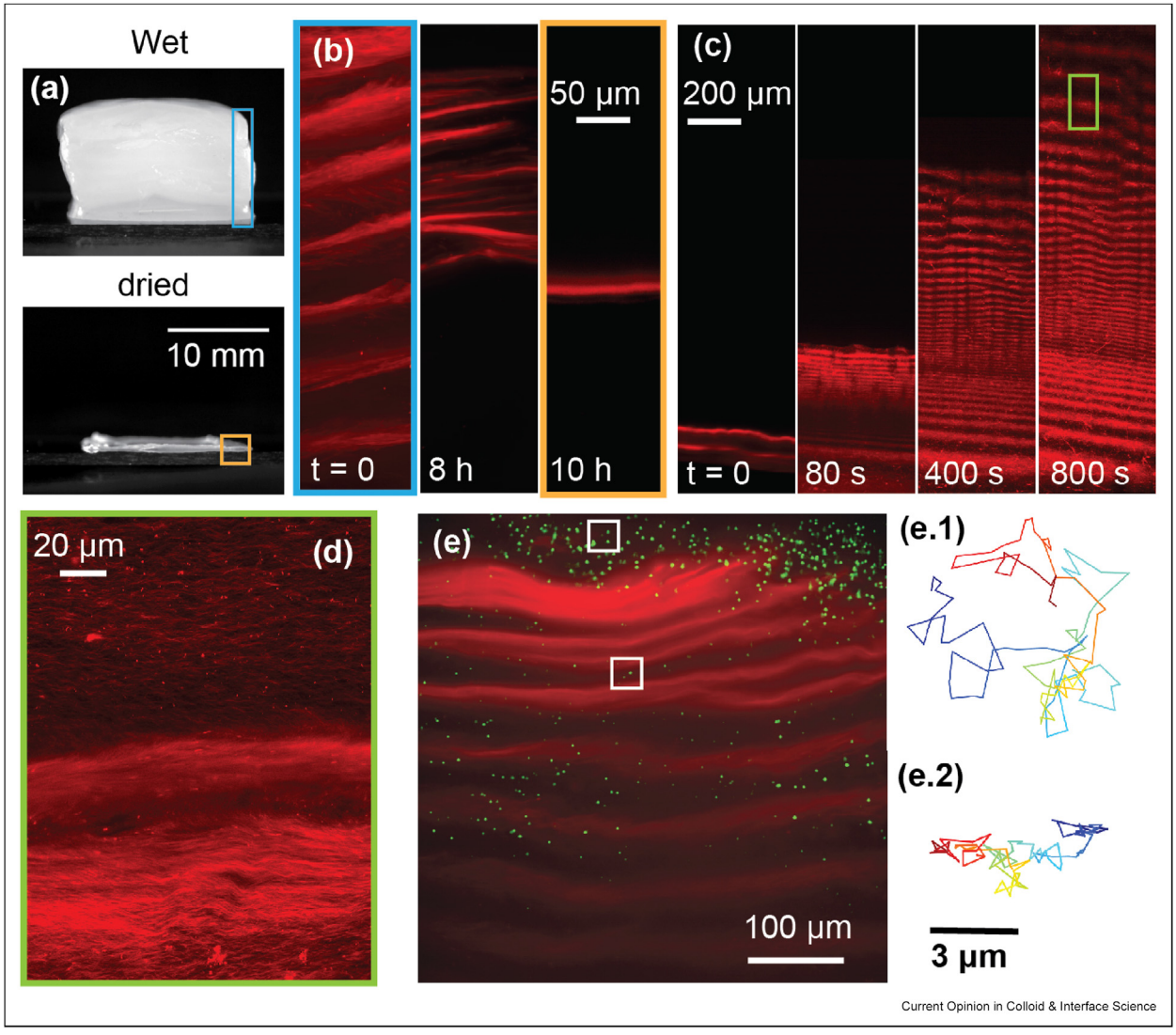
Hydrogel structure and swelling

Another common component of cosmetics, food, and consumer products is polymeric hydrogels: spongelike structures that adsorb large volumes of water and swell significantly to provide complex tactile response and other benefits. Consistent with the theme of LSFM providing multiscale characterization of soft structures in commercial products, the large-scale structure of hydrogels can be visualized and mapped in an LSFM experiment using concepts of the particle tracking microrheology community [33,27]. Particle tracking relies on the addition of fluorescently tagged colloidal tracer particles to a sample, then following the Brownian diffusion of the particles to assess their spatial and

temporal mobility. Where microrheology uses these measurements to assess fluid mechanical properties, in a hydrogel there is the opportunity to map the heterogeneity of the structure and its quantitative pore size distribution by assessing local and global variations in tracer mobility. LSFM here provides an advantage over other optical sectioning methods like confocal microscopy with its ability to map large volumes of samples with high heterogeneity, for example, hydrogels formed from bacterial cellulose [14] or other biologically-derived hydrogels. Using an objective with a high numerical aperture will provide high-volume imaging with the fast scanning required for fast particle tracking, along with enough resolution to capture 500 nm tracer particles, green in Figure 4e, and submicron cellulose fibers, red in Figure 4e, across the structure of the entire gel.

Figure 4e1 and e2 show the level of detail that can be obtained by tracking the diffusion of the tracers within the hydrogel. Figure 4e1 is the trajectory of a particle outside of a pore region, evidenced by the random

Figure 4



(**a**) Bulk imaging of the structural transformation of the bacterial cellulose film during drying, (**b**) from lightsheet imaging of the drying process over time, to (**c**) swelling of a dried sample and recovery of the layered structure, to (**d**) high-resolution confocal imaging of the fibrous network between the layers. (**e**) Bacterial cellulose fibers stained with Congo Red in a large biofilm are imaged simultaneously with green fluorescent colloidal tracers. Tracking the tracer trajectories allows for quantitative mapping of the local pore dimensions and the heterogeneous structure and mobility of particles where diffusion is free and homogeneous (e.1), and constrained and anisotropic (e.2).

motion visible in all directions, whereas Figure 4e2 shows the trajectory of a tracer particle that is constrained in an anisotropic pore environment. Carrying out this mapping across the entire hydrogel provides a full map of its regions of homogeneity and heterogeneity over a millimeter-scale soft product microstructure [14].

In addition to the porous structure provided by hydrogels to products, their significant swelling is also important to product performance. An example of the same bacterial cellulose hydrogel discussed above is shown in Figure 4a, where the dried form thickness is on the order of microns thick but then swells to more than 20 mm thick when hydrated. Figure 4b shows the process of drying, allowing assessment of the structural stability during extreme compression of the matrix over time as the gel goes from millimeters in thickness to microns. Similarly, dynamic processes like swelling are critical to product performance in cosmetic structuring, active delivery, and oil adsorption, while products like diapers and other absorbents rely on swelling to deliver their main product benefit. LSFM also enables characterization of the swelling process across the relevant length scales, illuminating how the hydrogel's microstructure expands and whether the hydrogel can withstand the capillary and osmotic pressure during swelling. For example, Figure 4c shows a series of LSFM images of the hydrogel in Figure 4a swelling after contact with water over several orders of magnitude in size. The multilayered structure of the mesh is visible as well as the spacing of the individual layers with time and hydration. The internal layers are the last to expand, as expected given the need for water to permeate the full volume. Figure 4d shows a zoomed-in CLSM view of the different mesh layers as a complement to the larger-scale characterization provided by LSFM.

Meat and alternative protein

The imaging of biological tissue is an already-optimized use of LSFM thanks to the technique's broad activity in the biological sciences. An incredibly active research area that can also benefit from this capability is the development of alternative protein [34] and cultured meat [35] products as replacements for resource-heavy animal-based proteins that are unavailable to vegetarians.

The lightsheet microscopy technique, combined with image analysis, can be used to investigate the structural transformation of meat tissues. Light-sheet microscopy allows fast imaging of large specimens in three-dimensions over time. This technique enables the three-dimensional imaging of different components, and computational image analysis provides a quantitative understanding of the physical and chemical variations in the structure caused by changes in environmental factors. The developed methodology and quantitative analysis can be used as a unique tool to map the structure of other tissues and to mimic their various characteristics.

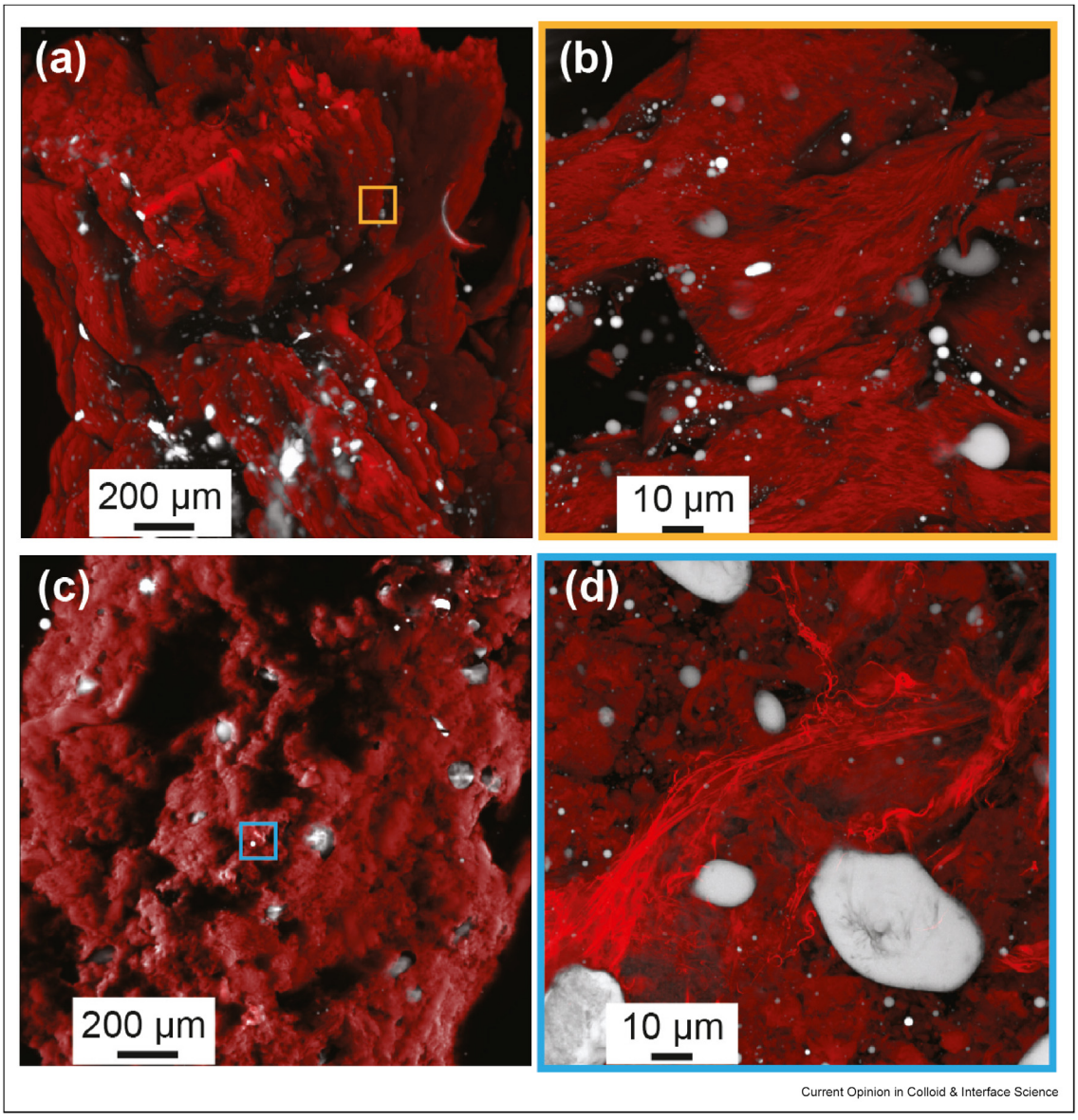
Comparison of a 3D reconstruction of a soy-based meat sample, Figure 5a, with a confocal section of the sample, Figure 5b, demonstrates the capabilities of LSFM imaging. The technique allows for imaging large samples and enhances the ability to analyze the distribution of components in the manufactured plant-based meat, directly affecting product quality and consumer perception. This method can be complemented by confocal microscopy, which provides a higher resolution of a smaller field of view and allows study of the distribution of submicron-fiber structures, for example, Figure 5b. Also visible are other components like white fat droplets that contribute to different aspects of texture and use for the consumer. As an aid to the design of complex fluid products like alternative protein meat, the broader structural overview provided by LSFM can be benchmarked against beef to compare the distribution of components and understand how the complex 3D structure contributes to the final texture of the product.

Comparison of the soy-based product in Figures 5a—b with a sample of beef mince meat in Figures 5c and d provides a clear comparison of the degree of organization and distribution of components in each case, allowing correlation with bulk mechanical properties that enable assessment of the most important elements for product quality. An example is the more complex hierarchical fibrillar structures produced by animal tissues that are visible in the confocal image, Figure 5d. Beyond the above examples of product structural and dynamic analysis, the speed and breadth of LSFM offer other novel benefits to product technologies. Starting from structural mappings like those in Figure 5, the design of new food and tissue structures could be created using the precision of LSFM images to draw three-dimensional structures in photopolymerizable polymers [36].

Future directions

As central as microstructure is to commercial and biological materials, so too is flow to the creation, modification, and response of complex fluid materials [1,2]. Superimposing flow onto imaging and structural studies using confocal microscopy [37,38], SAXS [39], and small-angle neutron scattering (SANS) [40] have provided influential insights to experimentalists for new development efforts and theoretical validation. We anticipate the same sort of advantages from the application of LSFM to flow and related processes in consumer products and provide some specific examples below as potential inspiration.

Figure 5



(**a**) Lightsheet 3D reconstruction of a plant-based meat and (**b**) confocal image of the sample show the difference in scale between the two imaging methods. (**c**) Comparison of ground beef meat images using the lightsheet technique versus (**d**) confocal microscopy. Proteins are labeled red and lipids are labeled white.

Gel collapse

Delayed collapse of colloidal gels and, by extension, products stabilized by colloidal gel yield stresses [4,1], is a phenomenon that occurs when the weight of a gel's components or the material it suspends compresses the gel and exudes the bulk fluid [41]. As collapse and consolidation can lead to heterogeneous microstructures in a fluid product, the spatial variation of the structure is of significant interest and has been studied theoretically [42] and with MRI imaging [43]. For product instabilities, early detection is often a critical benchmark of a product's viability because of their long shelf life requirements. LSFM could use selective tagging of product ingredients to detect such structural failures on small length scales, even over a significant product volume, while also indicating the occurrence of any associated phenomena like segregation or phase separation. The results in Figure 4 are a good indicator of the level of detail possible in such a case.

Adaptation of flow systems to LSFM

Just as rheometers have been modified and developed for use in confocal microscopes [37,38] and SAXS [39], other flow geometries can be modified and used directly in the beam of a light sheet microscope [44,45]. The fluidic four-roll mill [5], for example, could be adapted to enable light sheet imaging of structural evolution with time and spatial variation as a mesostructural complement to the molecular and microscale insights provided by SAXS [5,39] measurements. It would also enable tuning of the degree to which shear or extensional flow deformation affect product structure and time evolution. The dripping on substrate, DOS, technique of extensional rheology provides another rapid measurement of flow properties of fluids [46] even at small volumes [47] and using only surface tension to drive flows. The test involves imaging the formation, thinning, and breakup of a fluid filament and comparing the deformation to fundamental models of key non-Newtonian rheology. The cylindrical geometry that forms is nicely compatible with the dimensions and form of LSFM samples and could even be performed inside of a standard sample tube. Matching the time scales of flow and structural response to exceed those of the LSFM method would enable three-dimensional analysis during flow. Given the importance of these flows to consumer products [48] and foods [49], the approach could extend the sorts of insights typically gained from shear flow imaging experiments. Other phenomena, like surface film formation during fiber spinning and product aging [50] could also be studied in much greater detail using a hybrid rheology-LSFM approach. While conventional DOS measurements explore fluids without a significant yield stress, a variation on the technique has recently been developed that enables measurement of yield stress [51].

Shear banding

Another application of simultaneous structural and flow studies is the examination of shear-banding and flow heterogeneity effects [52]. Shear banding occurs when flow-induced heterogeneity appears in a complex fluid system, often near a solid surface. Shear banding can have a strong effect on rheological measurement accuracy [53] as well as the lubricating effects of complex fluids undergoing small-scale flows on a surface. As a result, the phenomenon is directly relevant to formulated product perception and performance during flow, such as spreading a cream onto skin. Flows like that balance rheological and tribological length scales are highly relevant to food properties like mouthfeel [54] as well as textural perception like lubricity [55]. Measurements of complex fluid structure, like small-angle x-ray scattering, SAXS, can also be biased by flow banding effects, as seen for viscoelastic liquid crystalline extrusion into SAXS beams to study protein crystallization [56]. It is likely that phenomena like shear banding will transition from theoretical inspirations and measurement frustrations to desirable product attributes, but their complex nature must be better understood using techniques that couple structure, flow, and multiple length scales, like LSFM.

Conclusions

There is a strong recognition of the need for increased understanding of complex fluid formulations and their structure—performance relationships. Many methods exist, with approaches like confocal microscopy and x-ray scattering contributing enormous insights to the field and associated theories of complex fluids. Light sheet microscopy offers a potential bridge to larger length scales, connecting the threads of product formulation, manufacture, and use and building on the strong foundations laid by confocal microscopy and x-ray scattering studies to enable mesoscale imaging more broadly.

As pointed out in earlier reviews [15], LSFM offers unique flexibility for external manipulation of samples by magnetic fields, rheometer geometries, and thermal treatments. Here we advocate for the extension of these ideas to the realm of commercial complex fluid products. Because many of the applied and industrial questions regarding complex fluid products rely upon perturbation to assess stability and performance, there will be new insights gained by further integrating this fascinating technique into the formulated product and high-throughput [57] lab toolkit. The open-source hardware and software communities that have developed around LSFM development and use [58] will enhance and accelerate the technique's adoption by academic and industrial researchers in the space of complex fluid formulations.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:
Patrick T. Spicer reports financial support was provided by International Fine Particle Research Institute. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This manuscript was derived from a plenary talk given by PTS at the International Fine Particle Research Institute, IFPRI, Workshop on Powder Reconstitution and we are grateful for their support that enabled

participation. We are also thankful for conversations with Prof. Eric Furst (U. Delaware) regarding the needs of academic and industrial researchers for new imaging methods of soft and complex microstructures. We are immensely grateful for the advanced capabilities and support of the imaging experts of the Katharina Gaus Light Microscopy Facility (KGLMF) at UNSW, especially Dr. Sandra Fok. Dr. Pavel Tomancak, Central European Institute of Technology, deserves special thanks for sparking our excitement in light sheet microscopy with a talk given at UNSW in 2017.

Data availability

Data will be made available on request.

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