## Opinion

## Soft matter is illuminated by light sheet fluorescence microscopy

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## INTRODUCTION

I n investigations of soft matter, volumetric microscopic imaging data taken at fast speeds is frequently required. There There are several microscopy methods available for this purpose, however light Sheet Fluorescence Microscopy (LSFM). Over the last two decades, this microscopy technology has grown dramatically in the biological sciences. In this context, we illustrate how LSFM may be used to the realm of soft matter. We cover how LSFM has been employed in previous soft matter research, as well as its principles and current improvements. We show how a recent LSFM implementation may be utilised to investigate capillary wave fluctuations and droplet coalescence in a colloidal fluid system.

Light Sheet Fluorescence Microscopy (LSFM) has a large and growing user base, primarily among life scientists. This is due to the LSFM's ability to acquire optically sectioned images while using minimal excitation light. Though light sheet microscopy has been around since the early twentieth century, work in the early twentyfirst century has seen a significant increase in its popularity. Notably, Jan Huisken and colleagues in Ernst H. K. Stelzer's lab demonstrated the utility of LSFM in imaging developing embryos over days without visible photo damage in 2004. Numerous LSFM designs have been published since then, pushing the technique's capabilities in a variety of directions ranging from increased spatiotemporal resolution to aberration correction.

The biological sciences have been the primary applications for these various LSFM designs. However, in this article, we highlight work in the field of soft matter that has used LSFM, describe recent advances in LSFM that may entice more scientists investigating nonliving materials to use LSFM, and describe results using LSFM on colloidal samples in our own lab.

There are numerous LSFM configurations, but the demonstrations of the technique that sparked its renaissance over the last two decades, and a configuration that is still used today, use two orthogonally placed objective lenses with the sample at the common focal point.

One objective lens, known as the excitation or illumination objective,

directs laser light to a thin sheet of sample. The other objective lens, known as the imaging or detection objective, has its focal plane aligned with this sheet of excitation light. Optically sectioned images can then be captured at the camera's frame rate, similar to wide field methods and faster than point scanning methods. Image stacks can be acquired for Three-Dimensional (3D) imaging by either translating the sample through the light sheet or sweeping the light sheet in sync with translation of the imaging objective's focal plane using, for example, a scanning mirror and a piezoelectric stage.

Innovative LSFM designs have expanded the range of applications where the technique excels far beyond imaging of living whole organisms in recent years. This was accomplished by allowing for greater flexibility in sample mounting procedures, increasing the speed of 3D image stack acquisition, and improving spatial resolution. While some of these LSFM advances are difficult to replicate without extensive optical engineering knowledge, an increasing number of LSFM configurations are commercially available. Furthermore, there are numerous open access platforms and guides available for researchers interested in building their own LSFM using mostly off-the-shelf components.

While the vast majority of LSFM research is in the life sciences, we believe the technique has great potential for soft matter and material scientists. Of course, those studying nonliving materials are less concerned with LSFM's low photo toxicity and photo bleaching. When working with colloids and emulsions rather than living cells and embryos, photons are not as scarce. Nonetheless, the spatiotemporal resolution and data throughput rates achieved by LSFM make it competitive with the laser scanning confocal microscope, the workhorse optical sectioning technique in many soft matter labs. Indeed, looking at the history of confocal microscopy's application in materials science and soft matter suggests that we may be approaching a tipping point.

The number of LSFM configurations has increased dramatically in recent years. Many recent designs apply the most recent concepts and methods from the larger field of optical microscopy to the light-sheet modality. Adaptive optics for aberration correction, fast focal plane movement with electrically tunable lenses, single-molecule

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localization microscopy, structured illumination, and multiphoton excitation, for example, have all been used to improve LSFM speed and/or spatial resolution. We will not go into the specifics of their implementation and use because many of these advances are applicable to multiple microscopy modalities, not just LSFM. Instead, we will focus on the more LSFM-specific advancements. The ideal shape of the excitation light sheet is thin, allowing for high optical sectioning and improved axial resolution over a wide lateral extent, resulting in a large field of view. However, the sheet's thinness and uniformity over a wide field of view are competing requirements. A thin light sheet can be achieved with a high Numerical Aperture (NA) illumination objective. However, there will be a limited lateral distance (i.e., distance along the propagation direction of the excitation light) over which the light sheet thickness can be approximated as uniform.