



Effect of humidity on liquid-crystalline myelin figure growth using digital holographic microscopy

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ABSTRACT

Dynamics of liquid-crystalline Myelin Figures (MFs) is a multifaceted issue depending on various elements, which have not been fully resolved yet. Our experimental results show that degree of humidity is influential on the initial growth and dynamics of MFs - a factor that was not been carefully considered on MF formations. In this paper, we present a systematic experimental study on the effect of humidity on MF dynamics. Quantitative analysis of MF dynamical behavior was performed using digital holographic microscopy (DHM). Our study reveals that humidifying the initial lipid reservoir has reverse effect on the rate of the growth to the extent that complete saturation of the lipid source prevents MF growth. The phenomenon is explained by the role of hydration gradient during MF formation.

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1. Introduction

There are numerous materials in the world with either understood/useful or unclear/strange behaviors [1–6]. Liquid crystals can be found frequently among materials with strange manners. For example, concentrated mesophases of some membrane-forming liquid crystals in water deform readily producing multilamellar cylindrical tubules known as myelin figures (MFs), via a close interplay of fluidity and elasticity. The formation of these complex liquid-crystalline structures and their dynamics are indeed fundamental, unresolved questions and challenging biological features for many researchers since their first observation in 1854 [7–13].

Although MFs can be formed in complex biological systems, their formation and organization bear strong similarity to those obtained during hydration of dried synthetic lipids by water. This in vitro model of MF has proved valuable in studying its fundamental features and dynamics.

Although there are some studies about the MF imaging [14,15],

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its structure [12,16,17], its initial growth rate [18,19] and coiling behavior [20–25], there are not much investigations explaining the reason of MF formation. However, there are some hypotheses in this regard including a report from Sakurai et al. in which the authors suggested that growth of MFs governs by the collective diffusion of surfactant in solution (diffusive growth model) [8]. Structurally, Buchanan et al. at 2000 hypothesized MFs develop from a blistering instability on a planar bilayer and grow in length as the dry surfactant absorbs water (growth-by-swelling mechanism) [26]. Zou et al., in a report at 2006, shows the previous models cannot cover all the facts regarding the growth and dynamics of MFs [27]. This report claims that MFs formation and growth relies on applying a driving stress. This driving stress can be the hydration gradient, which makes the effect of humidity important (such as the investigation of this paper). In this context any factor that influences the facilitation or elimination of the driving stresses will lead to a change on the MF dynamics. In this letter, we have considered the influence of humidity on MF growth and dynamics. Our experiments show that, besides other factors, growth and dynamics of MFs significantly depend on the degree of humidity in the reservoir of material from which the MFs protrude. However, to the best of our knowledge, this subject which is the focus of this paper, has not yet been systematically studied.

We have performed our quantitative analysis by the use of digital holographic microscopy (DHM) technique on lipidic structure. DHM in a transmission mode can be an effective tool for

quantitative visualization of phase objects such as living organelles [28–31]. By deriving phase information from the interference pattern between light wave passing through the lipid sample and a reference wave, DHM provides three-dimensional (3D) information on the morphology and volume of MFs with high resolution and at time scales from milliseconds to hours. It enables us to focus on different layers of our samples by numerical methods [32]. Hence, in comparison with conventional microscopy techniques, this method results in more precise and reliable information of MF growth.

Our DHM observation in this study indicated that the humidifying the lipid reservoir (lipid cake) during the incubation time prior to its contact with water can make major influence on the dynamics of MFs and they grow with different rate through lipid cakes with different degrees of humidity. The humidifying effect

can reach to the extent that complete saturation of the lipid reservoir prevents the MF growth.

2. Materials and methods

The lipid studied in this research, 1-Palmitoyl-2-Oleoyl-Sn-glycero-3-Phosphocholine (POPC), was purchased in powder type from Avanti and chloroform was used as the solvent. Throughout the experiments deionized water was used to initiate formation of MFs. Clean and dry glass slides and cover slip were used to construct the appropriate chambers for the experiments. Droplets with the volume of 1 μl of the solution were put on a glass plate. The samples were kept in vacuum overnight for chloroform evaporation, and then were incubated at 25 °C in a humid

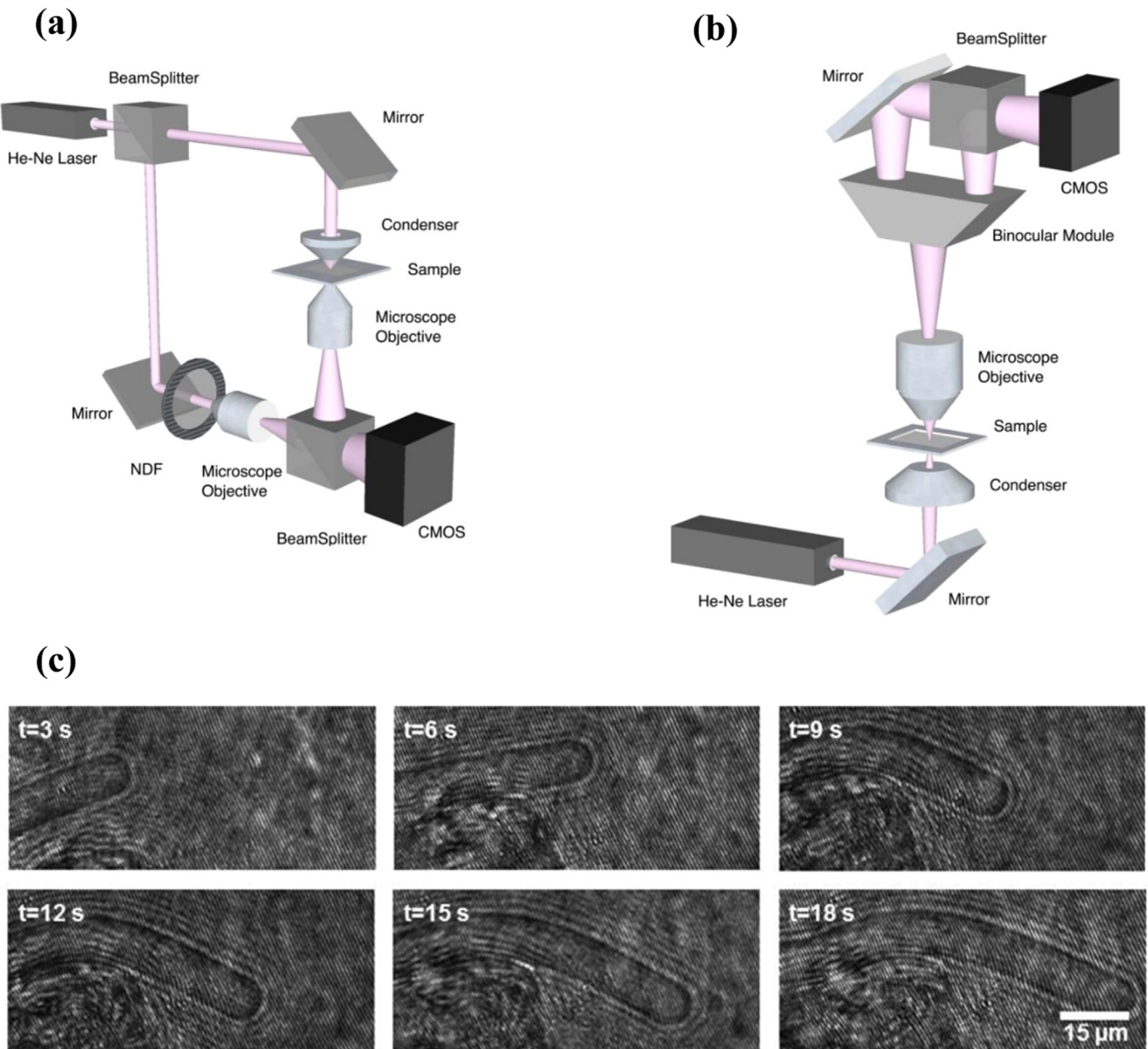


Fig. 1. Schematic DHM setups based on (a) Mach-Zehnder interferometer, (b) The improved self-referencing interferometry which was used for our experiment in this paper. The interference fringes are formed by superposition of a portion of the object beam on itself. The set up is placed on a binocular microscope and a He-Ne laser (MEOS, 632.8 nm, 5 mW) is used as the source. The beam passes through the sample and the objective (40X, Olympus), and by the binocular module, is split into two equal wavefronts with the same information of the sample. The two beams are recombined at a detector (Thorlab-DCC1545M) after inserting a slight angle in one of the beams to achieve off-axis holograms; (c): Recorded holograms of an MF at $t=3$ s to $t=18$ s after its formation starts.

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