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- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** Structural dynamics of laser excited lipid membranes	**Experiment number**: SC3406
Beamline: Id09B	**Date of experiment**: From: 16.05.2012 to: 20.05.2012	**Date of report**: 28.08.2013
Shifts: 15	**Local contact(s)**: M. Wulff, D Khakhulin	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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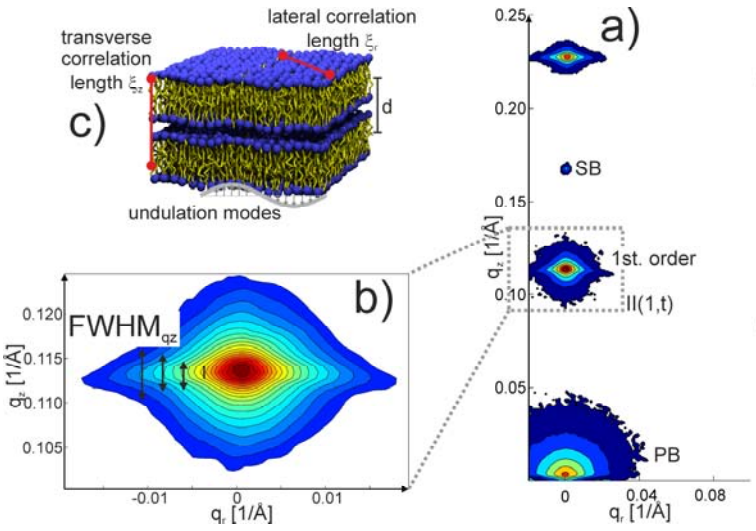
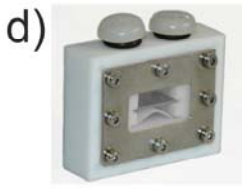
Report: We report on time resolved scattering experiments on fluorescently labeled lipid multilayers over a broad range of time scales (ns – μs). The structural and collective response after short pulse excitation by nanosecond laser pulses has been investigated in view of the non-equilibrium dynamics in lipid membranes. Following a very successful preceeding experiment (SC3150) during which thermal drift (induced by laser as well as x-ray radiation) could be minimized and suitable laser parameter regimes could be identified, we now observed non-equilibrium shape fluctuations in fluorescence labeled phospholipid multibilayers composed of the model lipid DOPC and the well known lipid dye Texas-red. It is found that pulsed laser illumination at moderate peak intensities ($P_p \leq 10^5 W/cm^2$) already leads to signifficant changes of the in plane membrane correlation length by up to 50% as well as the excitation of transient conformal undulation modes of a well defined lateral wavelength. The observed phenomena evolve on nano- to microsecond timescales after optical excitation, and can be described in terms of a modulation instability in the lipid multilamellar stack.

Materials and Methods: The experiments were carried out using monochromatic undulator radiation of 17keV photon energy (≈ 3eV resolution). This photon energy is well suited to penetrate the 10mm thick sample chamber containing the highly oriented 1,2-Dioleoyl-sn-glycero-3-phosphatidylcholine (DOPC) multilayers (N ≈ 1300 bilayers) containing 5 mole % Texas-red labeled DHPE (headgroup labeled) and deposited on quartz. Samples were fully immersed in a solution containing 30% wt. Polyethylene Glycol (PEG) (corresponding to an osmotic stress of 1.54MPa), preventing destabilization of the multilayers. The x ray beam incidence angle $\alpha_i \approx 0.5°$ was well aligned (GISAXS geometry) so that the specular reflected beam (SB) fell in between the first and the second diffraction order, see Fig.1 a), as recorded by the CCD detector (Frelon, ESRF). Stroboscopic data accumulation was carried out at a repetition rate of f ≈ 1kHz, using a pulsed laser system (Legend Elite, Coherent, Santa Clara, USA) synchronized to the 360'th harmonic of the electron bunch revolution frequency for optical excitation. The Nd:YLF Evolution pump laser inside the Legend Elite was used for optical pumping, operating at the second harmonic wavelength λ = 527nm, well matched to the edge of the absorption spectrum of Texas-red. The standard laser pulse duration was τ ≈ 180ns, while some data was recorded at increased temporal resolution of 5ns, roughly corresponding to the lifetime of the excited state, by further slicing the pulse down using a Pockels cell.
Characteristic changes in the membrane height-height correlation functions following short pulse excitation are analyzed by extracting fundamental length scales describing the collective membrane undulations as a function of delay after the optical stimulation from the diffuse scattering pattern (the measured structure Factor S(qz, qr), see Fig. 1 a,b) [1,2]: i.) The most important length scales are (see Fig. 1 c)) i.) the lamellar periodicity d, ii.) the rms-fluctuation amplitude σ, iii.) the in-plane correlation length ξ_r, and iv.) the the vertical correlation length $\xi_z(qr)$ which describes the correlation length in the direction z normal to the membranes, for an undulation with lateral wave number qr.





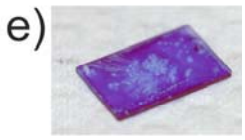
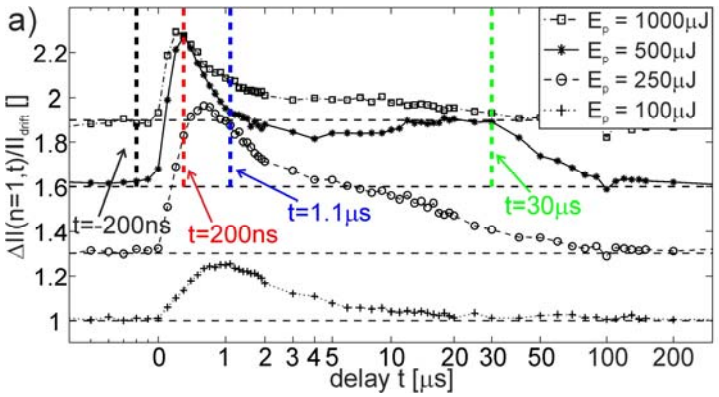


FIG. 1. (a) Example of a lamellar diffraction pattern with primary beam (PB), specular beam (SB), and the two first lamellar diffraction orders, with (b) a close-up of the first order reflection n = 1. From the two dimensional distribution, the lateral decay along qr and the width in direction of qz are extracted, to yield the temporal evolution of (c) lateral and vertical correlation lengths, respectively. (d) Sample chamber for oriented Texas-Red labeled lipid multilayers (e) fully immersed in solution.

Results: Fig. 2 a) shows the temporal evolution of the diffuse scattering intensity pattern highlighting the main changes in the lineshape for four different pulse energies Ep, followed by Fig. 2 b) which presents the relative changes in all observables (integrated intensity II(1,t), latteral correlation length ξ_r, lineshape parameter γ, smectic length scale $\Lambda \sim \xi_z$) extracted from the the first diffraction order (n=1) and the case of a laser pulse energy Ep of 250 µJ. A sharp intensity increase within the first 1µs after optical excitation is observed in all cases, the functional form of II(n, t) as well as the relative amplitude both depend on Ep as well as n. The rise time of the initial intensity increase shortens with pump energy, from t ~ 1µs for Ep= 100µJ to t = 100ns for Ep = 1000µJ. Relaxation to thermal equilibrium takes place on the 10µs time scale, and the system has fully relaxed after t = 100µs in all cases. As II(n, t) ~ $q^2\sigma^2$ for qz < 1, the fast intensity increase can be linked to an increased positional disorder σ of the membranes upon laser excitation. The intensity relaxation on the µs is attributed to the energy dissipationv via collective modes, possibly a collective undulation amplitude. The bilayer periodicity d(t) ≈ 53.6Å as determined from peak positions (center of mass) remains unchanged in all cases.



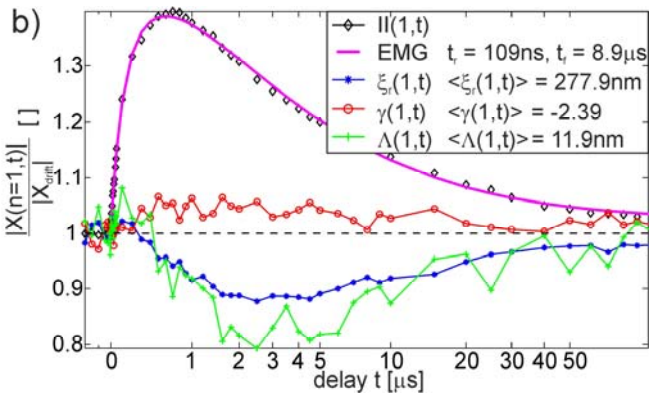


FIG. 2. (a) Evolution of the integrated intensity II(n = 1, t) for the first diffuse Bragg sheet at four different laser energies, curves corrected for drift and shifted for clarity. A steep increase on the 100ns timescale is followed by a relaxation on the µs timescale. Rise time and functional form of II(n, t) vary with applied pulse energy Ep. (b) Temporal evolution of the integrated intensity of the first order reflection II(1, t), lateral correlation length ξ_r(t), lineshape parameter γ(t) and smectic length scale Λ(t) ~1/ ξ_z(t) for laser excitation by pulses of τ = 5ns & Ep = 200µJ after normalization to the control (drift) measurements. The increase in diffuse intensity is followed by significant changes in the lineshape parameters, e.g. the smectic length Λ(t) as well as ξ_r(t) decrease to ≈ 85% within the first 2µs after excitation. Rise time t_r = 109ns and decay constant t_f = 8.9µs of the intensity increase are determined from least square fits of II(1, t) to an exponentially modified Gaussian distribution (EMG, solid, magenta).

Next, lineshape changes were investigated in addition to the intensity, see Fig. 2 (b) and Fig. 3, exploiting a strong reduction of the in plane correlation length ξ_r (i.e. a broadening of the diffuse reflections along qr) upon laser excitation. In particular the lateral momentum dependence of the structure factor S(n, qr, t) has

been analyzed in order to identify characteristic wavelength regimes on which the observed variations are most prominent, in other words to quantify the spectrum of relevant undulation modes see Fig. 3. A transient excitation of undulations in a well defined wavelength band is observed on the 1µs time scale. The central wavelength Γ = 138nm and bandwidth $\Delta\Gamma$ = 63nm as determined from non-linear least squares fits are independent of t. These observations can well be interpreted in terms of a modulation instability as predicted and observed [3] for tensile forces perpendicular to the bilayer normal, acting on a mechanically quenched system. In the present case, the absorption processes associated with the laser pulse lead to molecular fluctuations (quantifed by the observed increase in σ) which favor a larger lateral area per lipid and hence a smaller bilayer width. Since the mechanical response of the lipid film with a macroscopic thickness L and area is quenched on ns time scales (similar to [4]), internal stress builds up which can be relaxed by buckling without large scale mass transport.

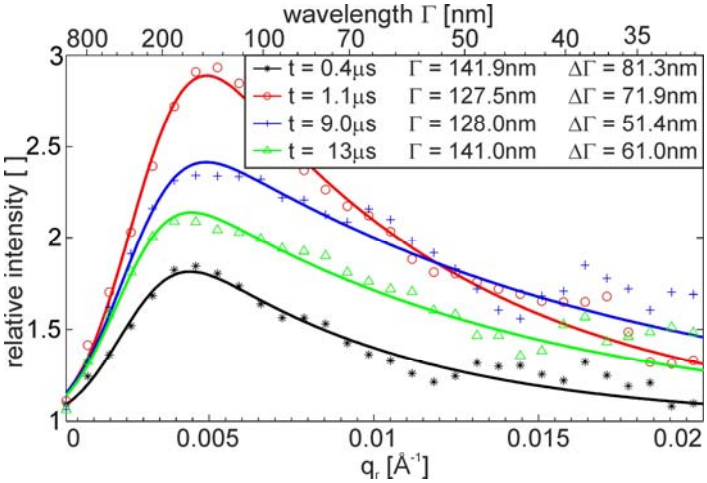


FIG. 3. Relative changes of the diffuse structure factor S(n = 1,qr, t) as a function of undulation wavelength Γ for four selected time delays t, illustrating the spatial frequency and the time scale of the observed changes.

Further analysis and a detailed discussion of our experimental findings which are relevant with regard to commonly applied fluorescent microscopy techniques, is in progress, and a full manuscript is in preparation.

References:

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