Multi-layered liposomes as optical resonators

Yong, Derrick; Ng, Wei Long; Lee, Elizabeth; Yu, Xia; Bosman, Michel; Chan, Chi Chiu

2013

Yong, D., Ng, W. L., Lee, E., Yu, X., Bosman, M., & Chan, C. C. (2013). Multi-layered liposomes as optical resonators. Progress in Biomedical Optics and Imaging - Proceedings of SPIE, 8598.

<https://hdl.handle.net/10356/99532>

<https://doi.org/10.1117/12.2002739>

© 2013 SPIE. This paper was published in Progress in Biomedical Optics and Imaging - Proceedings of SPIE and is made available as an electronic reprint (preprint) with permission of SPIE. The paper can be found at the following official DOI: [<http://dx.doi.org/10.1117/12.2002739>]. One print or electronic copy may be made for personal use only. Systematic or multiple reproduction, distribution to multiple locations via electronic or other means, duplication of any material in this paper for a fee or for commercial purposes, or modification of the content of the paper is prohibited and is subject to penalties under law.

Downloaded on 26 Apr 2023 20:42:57 SGT

Multi-layered Liposomes as Optical Resonators

Derrick Yong^{a,b,*}, Wei Long Ng^{a,b}, Elizabeth Lee^{a,b}, Xia Yu^a, Michel Bosman^c, Chi Chiu Chan^b

^aPrecision Measurements Group, Singapore Institute of Manufacturing Technology, Agency for Science, Technology and Research, 71 Nanyang Drive, Singapore 638075; ^bDivision of Bioengineering, School of Chemical and Biomedical Engineering, Nanyang Technological University, 70 Nanyang Drive, Singapore 637457; ^cAnalysis & Characterisation, Institute of Materials Research and Engineering, 3 Research Link, Singapore 117602

ABSTRACT

Multi-layered liposomes, comprising a concentric series of lipid bilayers – separated at fixed distances and compartmentalizing aqueous solutions of alternating refractive indices – are proposed as optical Bragg resonators. Seminal work focuses on the feasibility of successive encapsulations coupled with size-control via extrusion. Synthesis criteria for realization of these liposomes were subsequently discussed based on experimental observations. Numerical studies of the proposed structure showed discernible band gaps, qualifying their potential application in biological lasing.

Keywords: Liposomes, spherical Bragg resonator, multi-layered spheres

1. INTRODUCTION

Liposomes, first described by Bangham and Horne [1] in 1964, are closed vesicular structures comprising self-assembled phospholipid bilayers. These same phospholipids are identical to the major components found in naturally occurring bilayers – cell membranes – and has seen to their use as viable membrane models in many studies [2-5]. In addition, the amphiphilic nature of phospholipids have given liposomes the capacity of trapping water- and lipid-soluble compounds in their aqueous cores and phospholipid bilayers, correspondingly. This unique trait has enabled numerous novel applications in controlled drug delivery [6-12], gene therapy [13-15] and even as micro-reactors [16] and bioanalytical systems [17]. On top of which, studies have also been performed on the synthesis of uniquely-shaped [18], multi-layered [19-21] and nano-compartmentalized [19, 22] liposomes, mainly targeted at improving drug delivery techniques by prolonging retention, overcoming the many membrane barriers within cells and enabling the encapsulation of multiple actives.

A direction less explored, however, is the optical properties of these structured liposomes despite demonstration of lipid-based optical gratings [23-25]. Multi-layered liposomes (MLL), in particular, possess concentric layers of phospholipid membranes that are structurally analogous to spherical resonators consisting of multi-layered spheres. These resonators, which have been widely explored and demonstrated to be capable of confining light at a particular wavelength with high Q-factors [26-28], fundamentally bank on the Bragg reflection or band gap effect created by the periodically alternating refractive indices of its dielectric layers. Additionally, these optical properties can be tuned by manipulation of the refractive index profile and periodicity of the multi-layered structure. This, together with the earlier highlighted encapsulating and compartmentalizing properties of liposomes establishes the feasibility of achieving optical resonance in MLL. Notably, the recent remarkable demonstration of biological lasers by Gather and Yun [29, 30] was highlighted by the authors to require miniaturization of its external resonators in order to facilitate the realization of *in vivo* lasing. The biological nature and inherent ability for self-assembly of phospholipids thus renders them potentially suitable for such an application, whilst not interfering with cellular integrity or possessing any bio-toxicity. Moreover, recent demonstration of cholesteric micro-lasers [31], bolsters the feasibility of using organic components in the creation of optical components.

This paper hence focuses on the synthesis of MLLs, which comprise of periodically separated phospholipid bilayers, size-controlled by extrusion. It further discusses criteria essential for the realization of the structure. Numerical studies were also performed to analyze the feasibility of the proposed MLLs as optical resonators. The eventual aim of this work would thus be to achieve a Bragg reflection or band gap effect in the synthesized MLLs.

*ZYYONG001@NTU.edu.sg; phone +65 67932072; fax +65 67916377

Bioinspired, Biointegrated, Bioengineered Photonic Devices, edited by Luke P. Lee,
John A. Rogers, Seok-Hyun Yun, Proc. of SPIE Vol. 8598, 859803 · © 2013 SPIE
CCC code: 1605-7422/13/\$18 · doi: 10.1117/12.2002739

2. MATERIALS AND METHODS

2.1 Chemicals and other materials

1,2-dipentadecanoyl-sn-glycero-3-phosphocholine (15:0 PC) were purchased from Avanti Polar Lipids Inc. (Alabaster, Alabama). Whatman NucleoporeTM track-etched polycarbonate membrane filters with varying pore sizes were obtained from GE Healthcare (Singapore). Phosphate buffered saline powder (pH 7.4 at 0.01M) was purchased from Sigma-Aldrich (Singapore). Formvar/Carbon 300 Mesh copper grids were purchased from Electron Microscopy Sciences (Hatfield, Pennsylvania). All other chemicals were of reagent grade.

2.2 Liposome synthesis

Liposomes were synthesized via a procedure based upon the preparation of reverse-phase evaporation vesicles (REV) previously described by Szoka *et al.* [32] and Pidgeon *et al.* [33, 34]. The aqueous and organic phases comprised phosphate buffered saline (PBS) and 15:0 PC dissolved in diethyl ether, correspondingly. Synthesized liposomes were extruded 10 to 15 times through a polycarbonate membrane. The result is a homogeneous suspension of small unilamellar vesicles.

To encapsulate the above-prepared liposomes in another phospholipid bilayer, aliquots of the liposome suspension was introduced as the aqueous phase to another volume of diethyl ether-dissolved lipids. The aqueous-in-organic mixture was processed similarly to the above. This hence results in a double-layered liposome with a desired layer-layer separation distance.

2.3 Size and concentration determination

The average size and poly-dispersion index (PDI) of the liposome suspensions were determined by dynamic light scattering using a Malvern Zetasizer Nano ZS. All readings were performed with approximately 0.5ml of samples in low-volume disposable cuvettes at 25°C.

Concentration of the samples was further quantified with NanoSight's LM10 coupled with its Nanoparticle Tracking Analysis software, which identifies particles as individual point-scatterers moving under the influence of Brownian motion. On top of the concentration, the system is similarly able to quantify the sample's particle size distribution. All readings were performed with approximately 0.3ml of samples.

2.4 TEM sample preparation

A small droplet of sample was first placed on clean piece of parafilm, where a copper grid was then floated on for about 3min. Upon removal of the copper grid from the droplet, the bulk of the adhered droplet was removed by gently blotting with a piece of filter paper. The remaining sample was then allowed to air-dry completely before being floated on a droplet of 1% uranyl acetate for 1min. Likewise, the excess was removed while the remainder was allowed to air-dry for at least a day before imaging with a transmission electron microscope (Titan) at 200kV.

2.5 Numerical simulations

A series of concentric spheres, analogous to the proposed MLL, was numerically studied for its optical properties. The structure comprises alternating layers of high (n_H) and low (n_L) refractive indices corresponding to aqueous solutions (6.01M/26% by wt. Sodium Chloride – $n_H=1.38$ [Mettler Toledo]; 15.35M/84% by wt. Sucrose – $n_H=1.50$ [Mettler Toledo]; and various Proteins – $n_H\approx 1.60$ [35]) and water – $n_L=1.33$. The layers are 100nm in thickness, conforming to the pore-size differences of the polycarbonate membrane filters, eliciting an n_H - n_L periodicity (Λ) of 200nm. Refractive index of the external environment corresponded to that of water. An input light source resided-in and spanned the core of the multi-layered structure, with an accompanying detector positioned tangentially to the top surface of the outer most sphere. Numerical simulations were performed with Lumerical's finite-difference time-domain method, over the wavelength span of 350 to 850nm.

3. RESULTS AND DISCUSSION

3.1 Synthesis and characterization of MLLs

Figure 1. Schematics of a multi-vesicular liposome undergoing extrusion through a polycarbonate membrane filter with pore size d , producing a double-layered liposome with a defined layer-layer separation of $\Lambda/2$.

The adopted synthesis process banks fundamentally on the immiscibility between the selected aqueous and organic phases as well as the self-assembly properties of phospholipids at these aqueous-organic interfaces, allowing for the encapsulation of aqueous-suspended liposomes by larger assemblies of lipid bilayers (multi-vesicular liposome, MVL). Following which, extrusion through a desired pore sizes results in defined layer-layer separation distances (equivalent to one half the periodicity) as shown in Figure 1. The realization of these MLLs (with defined layer-layer separations), however, can only truly be achieved when the following conditions are met: (1) liposomes to be encapsulated are of identical sizes and are homogeneously distributed; (2) amount of enveloping lipids in the MVL is sufficient to form continuous bilayers around all its encapsulated liposomes upon extrusion; (3) extrusion of MVLs has little to no effect on encapsulated liposomes; (4) empty liposomes formed during the formation or upon the extrusion of MVLs can be completely removed.

Current work mainly encompasses the synthesis and observation of consecutive encapsulations, where a maximum of 3 layers has been attempted with extrusions performed through pore sizes of 0.1, 0.2 and 0.4 μm . The requirement of homogeneity as depicted in conditions (1) and (4), would be addressed in future work using dialysis [37] and centrifugation [38] techniques mentioned in literature. Currently, only repetitive cycles of extrusion have been employed to improve the homogeneity of liposome suspensions as verified by size and PDI data (obtained from ZetaSizer), which of cause did not remove smaller sized liposomes. On the other hand, particle count estimations (obtained from NanoSight's Nanoparticle Tracking Analysis software) have been employed to meet condition (2), where the required input of encapsulating-lipids was subsequently estimated as follows:

$$\#lipids = n\pi \frac{d^2 + (d - 2t_{bilayer})^2}{A_{headgroup}} \quad (1)$$

Here, n – number of liposomes to be encapsulated; d – desired diameter of encapsulating-liposome; $t_{bilayer}$ – thickness of lipid bilayer; $A_{headgroup}$ – area of phospholipid headgroup. In brief, the amount of lipids was obtained by calculating the number of lipids residing in the outer and inner halves of the bilayer based on the surface area occupied by each individual phospholipid's headgroup in either halves. Nevertheless, this only provided the minimal amount of lipids required, and does not account for the lipids that form empty liposomes during the MVL formation or its ensuing extrusion, as highlighted in condition (4). Furthermore, the packing density within these multi-vesicular liposomes is

likewise a factor requiring consideration, where extruding a too tightly- or loosely-packed MVL may result in rupture or excessive amounts of empty liposomes respectively. Hence, to facilitate the accomplishment of condition (2) optimization of the encapsulating-lipids' input quantity as well as formulation of techniques for controlled MVL formation would be studied in future works. Apart from these, it is important to also highlight the temperature requisite of extrusion – above the lipid's T_m – as elastic deformation and subsequent budding can only be achieved when lipid bilayers are fluid [36]. However, as 15:0 PC comprised both the encapsulating and encapsulated liposomes, heating was executed with extreme caution and at moderation to minimize undesired gel-to-liquid phase transition of inner lipid bilayers. This was observed to result in reduced homogeneity and multiple closely packed layers resembling previously described multi-layered vesicles, which would be discussed later in this section. Consequently, it was essential that successive encapsulating layers consist of lipid mixtures with T_m lower than that of the previous, facilitating the extrusion-based sizing of the outer most lipid bilayer while maintaining the integrity of the adjacent inner layer. A graded T_m across the multi-layered structure would hence be subsequently explored, with the inner most bilayer having the highest T_m and thus thermal stability.

Figure 2. STEM micrograph of MVLs (a) and TEM micrographs of MLLs (b) & (c).

Figure 2 (a) further substantiates the earlier mentioned considerations regarding packing density and empty liposome formation, where each MVL is observed to encapsulate a substantial amount of liposomes amidst a significant population of empty liposomes. Figure 2 (b), on the other hand, likely depicts a 2-layered MLL, with inner and outer lipid bilayers marked with red arrows. The MLL here appears as 2 concentric spheres, with the inner one appearing darker – corresponding to a thicker region of overlapping lipid bilayers. It is noteworthy that the liposomes appear darker than the background, despite the negative stain employed, due to the phosphate-binding affinity of the stain. In addition, the distinct white ring around the MLL as well as its adjoining “spikes” is presumably drying-induced cracking of the residue, which itself was formed either from PBS's salt residue or interaction between its phosphate ions and the stain. In particular, as the outline of the MLL closely resembles that of their encircling white ring, it is possible that the crack formation was elicited by shrinkage of either the MLL or residue. This further implies that the hydrated diameter of the MLL might in fact be larger than that observed under TEM. It is thus important to use a fixing agent suitable for lipid bilayers, such as Osmium Tetroxide, in future sample preparations. Subsequent encapsulation of this MLL resulted in that shown in Figure 2(c). It is to be noted that the image here was obtained from a relatively thinner region, as reflected in the less homogeneous and lighter background. However, a graded series of 3 concentric spheres was not observed, probably resulting from a lack of discernible contrast between the innermost and adjacent layer. Despite this, an interesting series of concentric thin white rings were observed and were postulated to be the hydrophobic region of the bilayer (unaffected by the stain) that have appeared due a withdrawal of the external lipid bilayers upon drying. Its multiplicity, on the other hand, likely corresponds to closely packed lipid bilayers that often form during liposome synthesis – multi-layered vesicles. The layer-layer distances of these tightly packed layers have been reported to be of approximately 2.5nm [39], relatively insignificant as compared to the MLL layer-layer distances of 100nm – but definitely not negligible. Such features, although often removed during extrusion, was observed to be dominant in the prepared sample and attributed to the unfulfilled T_m as mentioned earlier. In addition, the observed displacement of the inner liposomes, as observed in both Figure 2(b) and (c), is speculated to be due to hydrophilic interactions between

phospholipid head groups that dominate upon the loss in water content upon drying. Observations from TEM micrographs thus highlight the importance of its sample preparation for accurate structural determination of MLLs, namely fixing and contrasting. Alternative imaging techniques, such as cryo-TEM, would likewise be explored to “capture” the MLLs in their hydrated states.

On top of achieving successive encapsulations, the feasibility of encapsulating and retaining various aqueous solutions of high refractive indices would likewise be further studied. It would comprise the devise or modification of synthesis methods for improving encapsulation efficiencies as well as the incorporation of additional components (such as cholesterol to the lipid bilayers) for prolonged retention.

3.2 Numerical simulations

Figure 3. Transmission spectra of 4 and 10 layered MLL for $n_L=1.33$ and varying values of n_H , namely 1.38 (sodium chloride), 1.50 (sucrose) and 1.60 (proteins). Inset: Schematics of 4-layered liposome depicting periodicity and alternation of high and low index layers as well as the numerical study layout.

With close considerations regarding the feasibility of encapsulation and potential bio-toxicity, aqueous solutions of sodium chloride, sucrose and proteins were analyzed in numerical studies with their respective refractive indices used as input parameters. Collected transmission spectra (as shown in Figure 3) illustrate a general drop in transmission with increasing wavelength and the presence of photonic band gaps spanning the 500 to 700nm region. In particular, the band gaps comprise 2 low transmission regions sandwiching a high transmission peak. This in fact corresponds to the existence of a quarter wavelength ($\lambda/4$) phase-slip [40] induced by the periodicity-disrupting core – 200nm in diameter, equivalent to the total thickness of two consecutive layers – that elicits a strong transmission peak amidst the Bragg-grating-induced band gap. It is to be highlighted that the band structure is advantageous towards the optical separation of absorption and emission bands – limiting and permitting their passage via low and high transmission regions corresponding – largely facilitating the requirements of lasing. Comparison between the transmission spectra of the 4- and 10-layered structures reveals significantly stronger band gaps for the latter, which can be attributed to the stronger reflectivity with the increasing number of alternating layers [41]. This, however, also emphasizes the importance of having sufficient layers before a significant enough band gaps can result, implying possible synthesis related limitations. On the other hand, the variation of n_H results in a general broadening of the band gap region upon its increase – an increase in difference with n_L [41]. Again, this similarly is similarly restricted by the highest attainable refractive indices of aqueous solutions. It is thus pertinent for further work to optimize these trade-offs. Nevertheless, the presence of distinctive band gaps demonstrates potential of MLLs for application as optical resonators.

4. CONCLUSION

This paper depicts the seminal work for the synthesis of MLLs as optical resonators. The proposed structure comprises a concentric series of lipid bilayers – compartmentalizing layers of aqueous solutions at alternating refractive indices and separated at defined layer-layer distances. Current work explores the feasibility of consecutive encapsulations and size-

control via extrusion. It also highlights the criteria for realization of MLLs, namely in attaining homogeneity, optimizing lipid input quantities, controlling formation of MVLS (the MLL precursor) and creating a T_m gradient across the series of bilayers. In addition, TEM sample preparation was similarly noted to require improvement for better visual determination of MLLs' structural features. Lastly, numerical studies of the proposed MLL stresses the importance of having ample alternating layers with significant refractive index differences, both of which are largely dependent on physical synthesis parameters. Its discernible band gaps, comprising 2 low transmission bands sandwiching a high transmission peak, is however promising for application as optical resonators.

ACKNOWLEDGEMENTS

This work is supported by the SERC TSRP Grant 1223600011. The first author would also like to thank the scholarship sponsorship by A*STAR Graduate Academy.

REFERENCES

- [1] Bangham, A. D., and Horne, R. W., "Negative staining of phospholipids and their structural modification by surface-active agents as observed in the electron microscope," *Journal of Molecular Biology*, 8(5), 660-IN10 (1964).
- [2] Sessa, G., and Weissmann, G., "Phospholipid spherules (liposomes) as a model for biological membranes," *Journal of Lipid Research*, 9(3), 310-318 (1968).
- [3] Bangham, A. D., Hill, W., and Miller, N. G. A., [Preparation and Use of Liposomes as Models of Biological Membranes] Plenum Press, (1974).
- [4] Masserini, M., Palestini, P., Pitto, M., Chigorno, V., and Sonnino, S., [Preparation and Use of Liposomes for the Study of Sphingolipid Segregation in Membrane Model Systems] Humana Press, 2 (2002).
- [5] Kaufmann, S., Kumar, K., and Reimhult, E., [Preparation and Dynamic Patterning of Supported Lipid Membranes Mimicking Cell Membranes] Humana Press, 28 (2011).
- [6] Zasadzinski, J. A., Wong, B., Forbes, N., Braun, G., and Wu, G., "Novel methods of enhanced retention in and rapid, targeted release from liposomes," *Current Opinion in Colloid & Interface Science*, 16(3), 203-214 (2011).
- [7] Drummond, D. C., Zignani, M., and Leroux, J.-C., "Current status of pH-sensitive liposomes in drug delivery," *Progress in Lipid Research*, 39(5), 409-460 (2000).
- [8] Mordon, S., Devoisselle, J. M., Begu, S., and Desmettre, T., "Laser-Induced Release of Liposome-Encapsulated Dye: A New Diagnostic Tool," *Lasers in Medical Science*, 13(3), 181-188 (1998).
- [9] Mackanos, M. A., Larabi, M., Shinde, R., Simanovskii, D. M., Guccione, S., and Contag, C. H., "Laser-induced disruption of systemically administered liposomes for targeted drug delivery," *Journal of Biomedical Optics*, 14(4), 044009-044009 (2009).
- [10] Paasonen, L., Laaksonen, T., Johans, C., Yliperttula, M., Kontturi, K. s., and Urtti, A., "Gold nanoparticles enable selective light-induced contents release from liposomes," *Journal of Controlled Release*, 122(1), 86-93 (2007).
- [11] Volodkin, D. V., Skirtach, A. G., and Möhwald, H., "Near-IR Remote Release from Assemblies of Liposomes and Nanoparticles," *Angewandte Chemie International Edition*, 48(10), 1807-1809 (2009).
- [12] Wu, G., Mikhailovsky, A., Khant, H. A., Fu, C., Chiu, W., and Zasadzinski, J. A., "Remotely Triggered Liposome Release by Near-Infrared Light Absorption via Hollow Gold Nanoshells," *Journal of the American Chemical Society*, 130(26), 8175-8177 (2008).
- [13] Gao, X., and Huang, L., "Cationic liposome-mediated gene transfer," *Gene therapy*, 2(10), 710-722 (1995).
- [14] Dachs, G. U., Dougherty, G. J., Stratford, I. J., and Chaplin, D. J., "Targeting gene therapy to cancer: a review," *Oncology research*, 9(6-7), 313-325 (1997).
- [15] Miller, A. D., "Cationic Liposomes for Gene Therapy," *Angewandte Chemie International Edition*, 37(13-14), 1768-1785 (1998).
- [16] Genç, R., Clergeaud, G., Ortiz, M., and O'Sullivan, C. K., "Green Synthesis of Gold Nanoparticles Using Glycerol-Incorporated Nanosized Liposomes," *Langmuir*, 27(17), 10894-10900 (2011).
- [17] Edwards, K. A., Bolduc, O. R., and Baeumner, A. J., "Miniaturized bioanalytical systems: enhanced performance through liposomes," *Current Opinion in Chemical Biology*, 16, 444-452 (2012).

- [18] Zidovska, A., Ewert, K. K., Quispe, J., Carragher, B., Potter, C. S., and Safinya, C. R., [Chapter 6 - Block Liposomes: Vesicles of Charged Lipids with Distinctly Shaped Nanoscale Sphere-, Pear-, Tube-, or Rod-Segments] Academic Press, (2009).
- [19] Sun, B. J., Shum, H. C., Holtze, C., and Weitz, D. A., "Microfluidic Melt Emulsification for Encapsulation and Release of Actives," *ACS Applied Materials & Interfaces*, 2(12), 3411-3416 (2010).
- [20] Akita, H., Kudo, A., Minoura, A., Yamaguti, M., Khalil, I. A., Moriguchi, R., Masuda, T., Danev, R., Nagayama, K., Kogure, K., and Harashima, H., "Multi-layered nanoparticles for penetrating the endosome and nuclear membrane via a step-wise membrane fusion process," *Biomaterials*, 30(15), 2940-2949 (2009).
- [21] Katayama, K., Kato, Y., Onishi, H., Nagai, T., and Machida, Y., "Preparation of novel double liposomes using the glass-filter method," *International Journal of Pharmaceutics*, 248(1,Äi2), 93-99 (2002).
- [22] Kisak, E. T., Coldren, B., and Zasadzinski, J. A., "Nanocompartments Enclosing Vesicles, Colloids, and Macromolecules via Interdigitated Lipid Bilayers," *Langmuir*, 18(1), 284-288 (2001).
- [23] Omkar, A. N., and Steven, L., "High-throughput optical quality control of lipid multilayers fabricated by dip-pen nanolithography," *Nanotechnology*, 22(22), 225301 (2011).
- [24] Lenhert, S., Brinkmann, F., Laue, T., Walheim, S., Vannahme, C., Klinkhammer, S., Xu, M., Sekula, S., Mappes, T., Schimmel, T., and Fuchs, H., "Lipid multilayer gratings," *Nat Nano*, 5(4), 275-279 (2010).
- [25] Nafday, O. A., Lowry, T. W., and Lenhert, S., "Multifunctional Lipid Multilayer Stamping," *Small*, 8(7), 1021-1028 (2012).
- [26] Ren, Z., Zhai, T., Wang, Z., Zhou, J., and Liu, D., "Complete Band Gaps in the Visible Range Achieved by a Low-Refractive-Index Material," *Advanced Materials*, 20(12), 2337-2340 (2008).
- [27] Liang, W., Xu, Y., Huang, Y., Yariv, A., Fleming, J., and Lin, S.-Y., "Mie scattering analysis of spherical Bragg "onion" resonators," *Opt. Express*, 12(4), 657-669 (2004).
- [28] Lin, L., and Chun, J., "Spherical Photonic Crystal Microcavity with Ultra-High Quality Factor." 1-3.
- [29] Gather, M. C., and Yun, S. H., "Single-cell biological lasers," *Nat Photon*, 5(7), 406-410 (2011).
- [30] Gather, M. C., and Yun, S. H., "Lasing from *Escherichia coli* bacteria genetically programmed to express green fluorescent protein," *Opt. Lett.*, 36(16), 3299-3301 (2011).
- [31] Humar, M., and Muševič, I., "3D microlasers from self-assembled cholesteric liquid-crystal microdroplets," *Opt. Express*, 18(26), 26995-27003 (2010).
- [32] Szoka, F., and Papahadjopoulos, D., "Procedure for preparation of liposomes with large internal aqueous space and high capture by reverse-phase evaporation," *Proceedings of the National Academy of Sciences*, 75(9), 4194-4198 (1978).
- [33] Pidgeon, C., Hunt, A. H., and Dittrich, K., "Formation of Multilayered Vesicles from Water/Organic-Solvent (W/O) Emulsions: Theory and Practice," *Pharmaceutical Research*, 3(1), 23-34 (1986).
- [34] Pidgeon, C., McNeely, S., Schmidt, T., and Johnson, J. E., "Multilayered vesicles prepared by reverse-phase evaporation: liposome structure and optimum solute entrapment," *Biochemistry*, 26(1), 17-29 (1987).
- [35] Mc, M. T. L., Groves Merton, L., and Hipp Norbert, J., [Refractive Indices of Amino Acids, Proteins, and Related Substances] AMERICAN CHEMICAL SOCIETY, 4 (1964).
- [36] Bouvrais, H., [Chapter one - Bending Rigidities of Lipid Bilayers: Their Determination and Main Inputs in Biophysical Studies] Academic Press, (2012).
- [37] Zhu, T. F., and Szostak, J. W., "Preparation of Large Monodisperse Vesicles," *PLoS ONE*, 4(4), e5009 (2009).
- [38] Güven, A., Ortiz, M., Constanti, M., and O'Sullivan, C. K., "Rapid and efficient method for the size separation of homogeneous fluorescein-encapsulating liposomes," *Journal of Liposome Research*, 19(2), 148-154 (2009).
- [39] Hope, M. J., Bally, M. B., Mayer, L. D., Janoff, A. S., and Cullis, P. R., "Generation of multilamellar and unilamellar phospholipid vesicles," *Chemistry and Physics of Lipids*, 40(2,Äi4), 89-107 (1986).
- [40] Bandyopadhyay, S., [Optical Properties of Solids] Springer US, 6 (2012).
- [41] Erdogan, T., "Fiber grating spectra," *Lightwave Technology, Journal of*, 15(8), 1277-1294 (1997).