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Pulsed laser deposition of organic and biological materials

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Abstract We report on the deposition of soft matter thin films by Matrix Assisted Pulsed Laser Evaporation (MAPLE). In particular, thin layers of biological material (Bovine Serum Albumin) and polymers (polyfluorene) for medical and optoelectronic applications, were realized by laser irradiating a frozen solution containing a low amount of material diluted in a laser absorbing volatile solvent. The depositions were carried out varying different parameters as solvent–solute concentration, solvent nature, laser fluencies, etc. The optical, morphological, structural and spectroscopical properties were detected by means of different analyses as FTIR, photoluminescence, AFM and SDS.

1 Introduction

Matrix Assisted Pulsed Laser Evaporation (MAPLE) is a novel physical vapour deposition technique able to deposit uniform polymer and biomaterial thin films over wide areas. MAPLE can be considered a variation of the conventional PLD and provides a more gentle mechanism for transferring many different compounds, ranging from small to large molecular weight species, from the condensed phase into the vapour phase. The MAPLE basic idea is the indirect way in which the laser energy is transferred to photosensitive materials, ensuring the preservation of the molecules integrity thanks to the solvent protective action [1, 2].

- The MAPLE setup is similar to the PLD one: in particular both employ a pulsed laser beam, a vacuum chamber, a pumping system, a substrate and a target holder. The main MAPLE features are: the target is usually made of a frozen matrix consisting of a diluted solution (0.1–5% in wt) of a polymeric, organic or biomaterial compound (solute) in a volatile solvent;
- The fluence values range from 50 to 500 mJ/cm^2;
- The solvent and the solute concentration must be chosen to form a homogeneous, particulate free solution;
- The solvent must be characterized by a high absorption at the working laser wavelength, high volatility and it has not to interact with the solute under.

As well the target is rotating during the evaporation process to avoid the rapid drilling of the target and a localized heating and melting of the frozen solution. The MAPLE process proceeds layer-by-layer, depleting the target of solvent and material in the same concentration as the starting matrix. When a substrate is positioned directly in the path of the evaporation process a coating starts to form, constituted by the evaporated molecules of the material. The volatile solvent molecules, which have very low sticking coefficient, are pumped away from the deposition chamber.

Finally, the ability to transfer polymers and biomaterials without significant damages is due to an optimization of different deposition parameters like: laser wavelength, fluence value, repetition rate, solvent, solute concentration,

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target temperature and background gas pressure into the vacuum chamber.

We deposited MAPLE by means of different materials. We report here the deposition of thin films of biological material (BSA), and polymers (polyfluorene).

2 Experimental setup

The experimental set up for MAPLE depositions is composed of several components. The source is a pulsed excimer laser, LPX 305i–Lambda Physik, operating at two different UV wavelengths: 193 nm (ArF) and 248 nm (KrF). The laser beam is focused, by a spherical lens ($f = 300$ mm) onto a target placed in a stainless steel vacuum chamber. The target temperature is kept at liquid nitrogen temperature thanks to a refrigerator target holder. A T type thermocouple (working range from −200 °C to +400 °C) is placed in contact with the reservoir of the holder near the frozen target to measure the temperature during the liquid nitrogen insertion into the reservoir and during the laser–solution interaction (Fig. 1). The laser pulse duration is 20 ns and the laser repetition rate is 10 Hz. The chamber is equipped with a pumping system formed by a rotative and a turbomolecular pump which can evacuate it down to a pressure of 10^{-4} Pa. Two vacuometers detect the pressure inside the chamber. A substrate holder is situated in front of the target and up to four substrates can be mounted for each deposition.

3 Experimental results

3.1 MAPLE depositions of BSA

Bovine Serum Albumin is the main component of blood proteins for all animals and plays a great role in the body. In detail, BSA is a midsized protein with a molecular weight of 66 kDa and an ellipsoidal shape with dimensions of 5 × 5 × 14 nm. The BSA protein solutions were prepared using two different solvents: deionized water and Phosphate Buffered Saline (PBS) which are typical solvents for diluting proteins. The preliminary experiments, based on laser light absorption, demonstrated that PBS solvent results the best choice when using the argon fluoride laser emission. The 193 nm wavelength was used also when water was utilized as solvent. The solutions, prepared with water and PBS, were gradually immersed in liquid nitrogen (−196 °C). When frozen, they were immediately mounted inside the vacuum chamber. The chamber was then evacuated down to few Pa by the pumping system. We varied different parameters as the solvent (water and PBS), the BSA concentration (1–2%) and finally the laser fluence

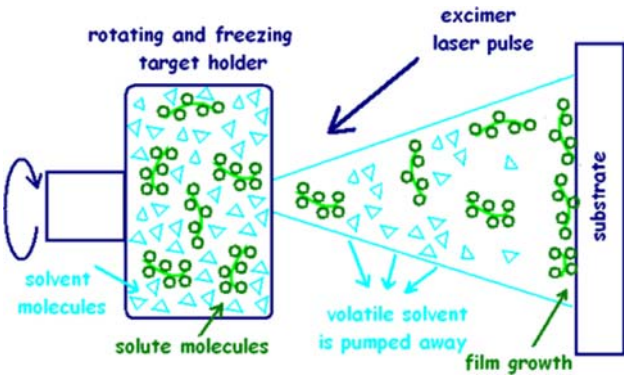

Fig. 1 Simplified scheme of the MAPLE desorption process

(in the range 75–250 mJ/cm^2) to check the quality of the deposited films and the onset of threshold effects in the deposition rate already observed for other systems. The spot size of the laser was 0.13 cm^2, the target-substrate distance 50 mm, the substrates wafer of <100> Silicon and all the films were deposited with 20,000 consecutive laser pulses. The deposited films are listed in Table 1.

Fourier Transform Infrared Spectroscopy (FTIR) was used to check if the molecular structure was preserved after the laser–target interaction; the spectra were recorded in transmission mode in the MIR range (800–5,000 cm^{-1}) with resolution of 4 cm^{-1}. In Fig. 2 the absorbance of the BSA films deposited with a fluence of 250 mJ/cm^2, starting from the solution of BSA diluted in water (#A1) and PBS (#S3), are shown together with the spectrum of a BSA bulk, for comparison. Each graph is the average result of 100 single acquisitions.

The spectra consist of the characteristic bands of the protein according to Ref. [3]. The major BSA absorption bands are present in the MAPLE BSA films at 1,653 cm^{-1} and 1,550 cm^{-1} and are usually called amide I (C–O stretching) and amide II (N–H bending), respectively. A minor band at 1,250 cm^{-1}, amide III, is less evident. With respect to these important wave numbers it is interesting to

Table 1 List of BSA samples with the different deposition parameters

Sample	Solution	Fluence (mJ/cm^2)
#A1	BSA (2% wt) in deionized water	250
#A2	BSA (2% wt) in deionized water	150
#A3	BSA (2% wt) in deionized water	75
#S1	BSA (2% wt) in PBS	150
#S2	BSA (2% wt) in PBS	75
#S3	BSA (2% wt) in PBS	250
#S4	BSA (2% wt) in PBS	150
#S5	BSA (2% wt) in PBS	500
#a1	BSA (1% wt) in deionized water	150
#s1	BSA (1% wt) in PBS	150

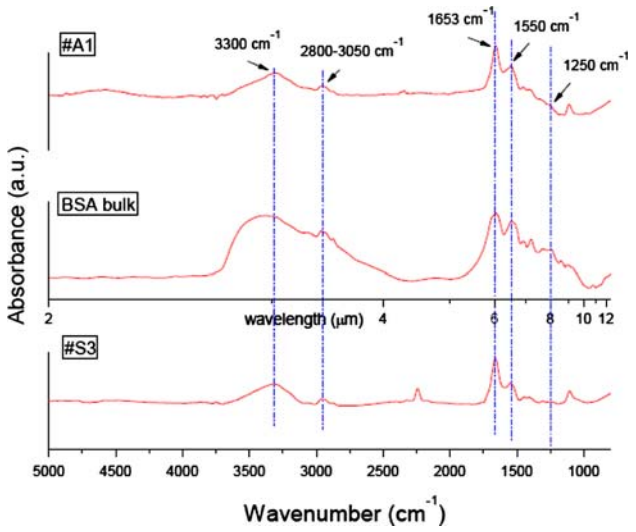

Fig. 2 Absorbance spectra of sample #A1, #S3 and BSA bulk

highlight that no red shift of these signals is present in the deposited films. It means that the secondary protein structure is preserved at α-helix state and no transformation in β-sheet configuration is formed after the laser irradiation [4]. This result confirms the absence of interaction of the protein molecules with the laser avoiding their denaturation. In fact, the β-sheet protein configuration should result in the presence in the FTIR spectrum of the shift of amide I to 1,628 cm^{-1} [5].

Atomic force microscopy was used to characterize BSA film surfaces. Roughness values, ranging from 2 (#A3) to 50 nm (#a1), were extrapolated by RMS analysis. Figure 3a shows a typical AFM image of a BSA film (#s1) deposited with a fluence of 150 mJ/cm^2 and a BSA concentration of 1% wt in PBS, while in Fig. 3b the 3-D reconstruction is reported. Similar structures are present in the other films.

The film is uniformly covered by circular shaped structures with dimensions from hundreds of nanometers to few microns. The well-known tendency of BSA to aggregate into macromolecular assemblies could explain the presence of objects of these sizes.

Biological tests were performed to confirm the protein integrity after MAPLE deposition. It is possible to determine the molecular weight of a macromolecule by using the sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) [6].

On the left of the Fig. 4, the molecular weights of BSA protein at different depth are listed. At the top it is reported the entire protein (66 kDa), while at the bottom the smallest protein fragment (14.2 kDa) can be detected. R indicates the column relative to the BSA protein used as reference, the second column regards the sample #S4 and the third one the sample #S5. For this kind of analysis the samples were deposited on KBr substrates which were then dissolved in a fixed water quantity. This solution was inserted into a gel, by using loading bores, to fabricate SDS-PAGE. In the second column no protein is revealed. It means that the film was made of an insufficient quantity of protein to be immobilized and detected, as a consequence of the low deposition rate due to the very low fluence value (150 mJ/cm^2) used. In third column only the band relative to the entire BSA protein is present, indicating that the fluence of 500 mJ/cm^2 is high enough to produce a reasonable rate without damaging the solute. The deposited BSA has an ellipsoidal shape with respect to the reference sample, due to the presence in the analyzed starting solution of KBr, which produces such a typical effect in SDS-PAGE.

3.2 MAPLE deposition of emitting polymer

Organic conjugated polymers (made by single and double alternated C–C bonds) are an important class of organic semiconductors. They are attracting considerable attention as a new material class to be used in photonic,

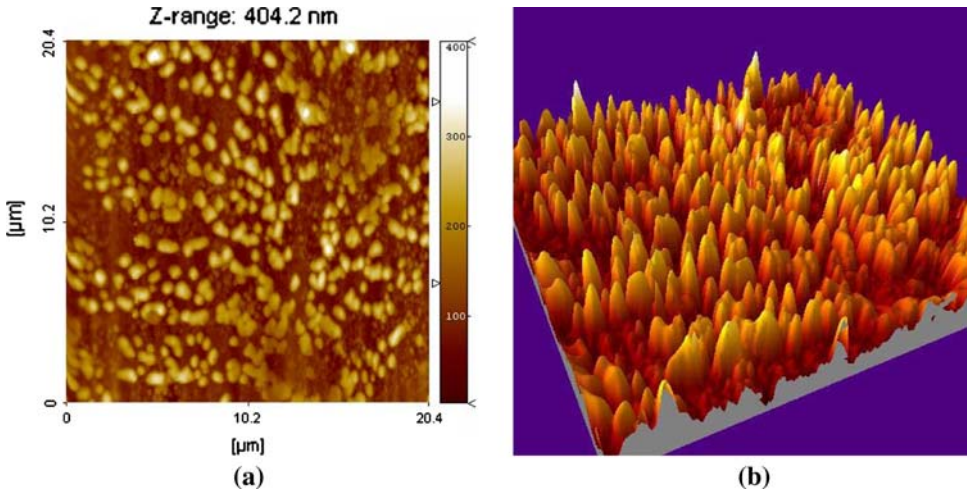

Fig. 3 (**a**) AFM and (**b**) 3-D extrapolation images of a BSA thin film (sample #s1)

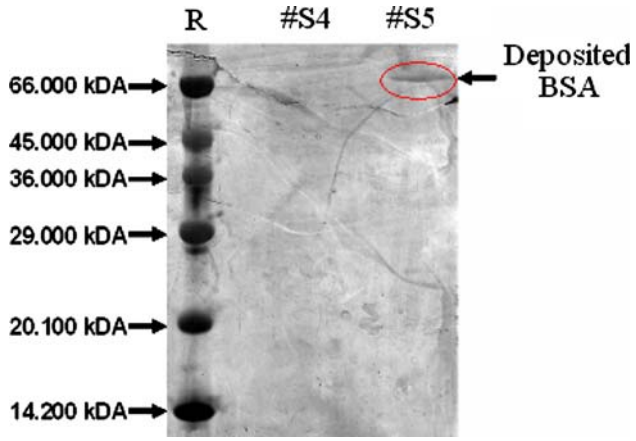

Fig. 4 SDS-PAGE image of pure BSA (R) and BSA films (#S4 and #S5)

optoelectronic and electronic devices. Especially, fluorene-based polymers have been recently employed to realize high luminance light emitting diodes [7, 8], photovoltaic devices [9] and thin film transistors [10]. To perform poly(9,9-dioctylfluorene) (PFO) depositions we used three different solvents with very low melting points: Chloroform, Toluene and Tetrahydrofuran (THF). A KrF excimer laser ($\lambda = 248$ nm; $\tau = 20$ ns) working at the repetition rate of 10 Hz was used. The influence of the different solvents and laser fluence on the PFO film properties was studied. The solution (PFO and solvent) was gradually immersed in liquid nitrogen (−196 °C) and, after complete solidification, quickly mounted on the cooled target holder, which rotated during laser irradiation to allow a uniform erosion. The target holder was pre-cooled at a temperature of −160 °C, before installing the frozen target, because of the low melting points of the used solvents. The chamber was evacuated down to 1×10^{-3} Pa. The target temperature, during the laser irradiation, was kept constant at −160 °C. The spot size was 0.058 cm^2, the target substrate distance was 36 mm and ‹100› silicon wafer was used as substrate. The deposited PFO films are listed in Table 2.

Table 2 List of PFO samples with the different deposition parameters

Sample	Solution	Number of pulses	Fluence (mJ/cm^2)
#P1	PFO in chloroform (0.5% wt)	10,000	90
#P2	PFO in chloroform (0.5% wt)	10,000	190
#P3	PFO in chloroform (0.5% wt)	10,000	50
#P4	PFO in toluene (0.5% wt)	5,000	200
#P5	PFO in toluene (0.5% wt)	5,000	350
#P6	PFO in toluene (0.5% wt)	5,000	500
#P7	PFO in THF (0.5% wt)	5,000	500
#P8	PFO in THF (0.5% wt)	5,000	350
#P9	PFO in THF (0.5% wt)	5,000	200

The samples were characterized by infrared spectroscopy (FTIR) in transmission mode in the MIR range (700–3,600 cm^{-1}) in which the polymer presents its typical features to evaluate the structural integrity of the polymer. Spin coated films were produced on the silicon substrates for comparison starting from the same solutions (chloroform, toluene, THF) utilized for the MAPLE depositions. These solvents are the ones which are generally used to dilute PFO for laser applications.

The comparison, as reported in Fig. 5, between the spin coated film spectrum with the ones of the samples deposited by MAPLE suggests that a chemical decomposition of PFO takes place when chloroform is used as solvent. In fact, the vibrational bands are broader, the relative intensities are not preserved and some peaks are missing. The chemical degradation of PFO deposited by MAPLE from chloroform, which is instead a good solvent in spin coating, is likely related to the presence of high reacting radicals produced by the $CHCl_3$ solvent decomposition under excimer laser irradiation [11]. It was also seen that the FTIR spectrum of the PFO film deposited using a toluene solution is quite noisy, but the characteristic PFO polymer peaks are all present. The signal at 1,100 cm^{-1}, which is ascribed to Si–O bond from the substrate, is slightly

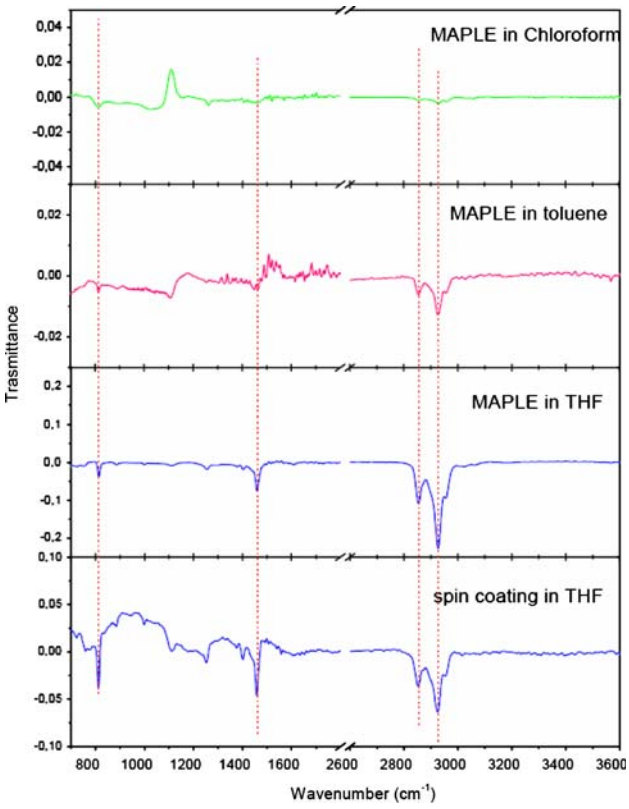

Fig. 5 FTIR transmittance spectra of films deposited by MAPLE with different solvents. A representative spectrum of a spin coated film is also shown

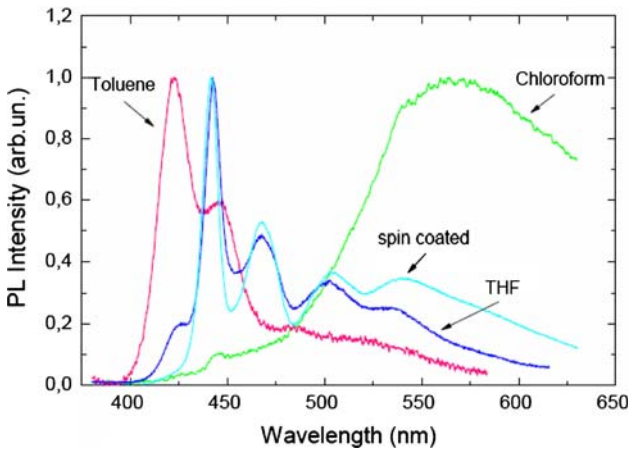

Fig. 6 Photoluminescence spectra of all the deposited samples

different from zero, due to a not perfect compensation of the substrate signal. On the contrary, the FTIR spectrum of the MAPLE deposited film using THF as solvent is very similar to the one of a spin coated film, since all the main peaks are present, preserving the relative intensities: the C–H stretching vibrations of the alkyl chain at 2,855 and 2,926 cm^{-1}, the C–H stretching mode of the aromatic ring at 2,953 cm^{-1}, the aromatic ring breathing vibration at 1,460 cm^{-1} and the alkyl C–H rocking mode at 813 cm^{-1}. Minor bands at 1,402 cm^{-1} and 1,377 cm^{-1} and at 1,253 cm^{-1} are also present, corresponding to alkyl chain C–H bending and alkyl chain C–H twisting modes, respectively [12, 13].

With THF and toluene as solvents, the raising of the laser fluence has no appreciable effect on the peak presence or position, but an increasing of the signal to noise ratio is observed due to an increase of the films thickness.

The effects of the deposition parameters on the film optical properties were investigated by Photoluminescence measurements (PL). The continuous PL measurements were performed with a He–Cd laser ($\lambda = 325$ nm). The samples were excited at room temperature with a power density of about 40 W cm^{-2}. The consequent PL emission was collected by a Si–CCD detector. The measurements were performed under vacuum, to avoid the sample photo-oxidation. PL spectra of PFO films deposited by MAPLE with different solvents were compared with the one of a spin coated film produced using toluene as solvent as reported in Fig. 6.

In the photoluminescence spectrum of the spin coated sample a main peak at 442 nm, typical of the 0–0 line of the PFO β-phase [14] can be observed, followed by vibronic replicas at about 476, 503 and 540 nm. The β-phase configuration in spin coated film is due to the evaporation speed of the solvent interacting with the PFO molecules during the spinning process. Moreover, the PL lineshape for wavelengths higher than about 500 nm suggests that fluorenone photoluminescence, typically peaked at about 570 nm and with a full with half maximum of about 180 nm [15], is overlapped to the pristine PFO PL. As the PL measurements are performed in vacuum, this suggests that PFO photo-oxidation takes place during the sample preparation in air. As regards the MAPLE deposited samples, the PL spectrum of the film obtained from chloroform solutions is dominated by an extrinsic defect band peaked at about 570 nm, more than 150 nm broad, very similar to the fluorenone emission, with a further weak peak at about 440 nm, due to the PFO β-phase 0–0 line. The strong defect emission is consistent with the FTIR results which showed a spectrum missing of the typical PFO vibrational bands. Therefore also PL result suggests a chemical degradation of the PFO, likely induced by reacting radicals produced by the $CHCl_3$ decomposition under UV light. The PL spectrum of the MAPLE film deposited from toluene solution is instead similar to the PFO glassy phase spectrum, with a main peak at about 421 nm, followed by a clear vibronic replica at 445 nm and by two weaker higher order vibronic shoulders at about 480 nm and 515 nm. No evidence of emission defects is present. It is interesting to observe that, despite the use of the same solvent (toluene) the MAPLE film shows the PFO glassy phase emission, while the toluene spin coated film shows the β-phase emission. As the β-phase is formed in spin coated films from toluene due to an interplay between aggregation in solution and solvent induced chain planarization in the solid phase [16]. The absence of the β-phase emission in the MAPLE deposited film suggests that molecular aggregation does not occur and that negligible interaction between PFO and toluene vapor takes place during the deposition process, thus avoiding the vapor solvent induced β-phase formation. It can be highlighted that in the depositions from toluene solutions only the PFO molecules reach the target, while the solvent molecules are pumped away during the process. This suggests that the solvent can be co-deposited together with the solute if the solute-solvent interactions (usually quantified by the Hildebrand parameter δ [17]) are strong. Actually the δ value for toluene is among the lowest ones for PFO solvents. This is consistent with weak PFO–toluene solvent interaction and indicates that the effective separation of the PFO molecules from toluene molecules occurs during the deposition process.

The MAPLE deposited film using THF as solvent shows a superposition of the emission features of the glassy and the β-phase, with a shoulder at about 420 nm (glassy phase) and a main peak at about 442 nm, due to the β phase, followed by vibronic replicas up to the forth order at 467, 502, 537 and 570 nm. It is important to stress that no defects emission are present. This feature confirms the

absence of interaction between the PFO molecules and laser irradiation. Moreover, the good vacuum condition avoids the oxidation of PFO, which in contrast characterizes the spin coated films. The spectrum is much more close to that of pristine PFO film with the added feature of the simultaneous presence of both configurations of the PFO molecule, with a prevalent contribution of the β-phase.

4 Conclusions

We proved that MAPLE is a suitable technique to realize thin films of so-called soft matter and that the quality of these films is, at least, comparable with other conventional methods.

In particular Bovine Serum Albumin thin films were deposited by Matrix-Assisted Pulsed Laser Evaporation. The primary and secondary structure of the protein was analyzed. A parametric study was carried out to find a good BSA solvent and the right deposition fluence values. From FTIR analyses, we obtained good quality films using as solvents, Phosphate Buffered Saline (PBS) and bi-distilled water. In fact, by comparison of the BSA bulk absorbance spectrum with the ones relative to the MAPLE deposited films, all the characteristic peaks were revealed confirming the integrity of the protein secondary structure. The spectra showed that the α-helix structure is dominant, as indicated by the absence of the peak related to the β-sheet protein configuration located at 1,628 cm^{-1}. The primary structure of BSA molecule was ensured from SDS-PAGE test. The film deposited on a KBr substrate and analyzed by electrophoresis gel revealed the presence of the BSA protein with the entire molecular weight even though these films were deposited at fluences higher than the ones usually employed in typical MAPLE depositions of biomaterials.

AFM images evidenced a uniform coverage of the substrate by the protein, which presented the typical spheroid-like BSA structures.

Poly(9,9-dioctylfluorene) thin films were deposited by Matrix-Assisted Pulsed Laser Evaporation using a KrF excimer laser. The influence of the laser fluence (50–500 mJ/cm^2) and the nature of different solvents (chloroform, toluene, tetrahydrofuran) on the film properties were studied. The chemical composition of the deposited films was investigated by Fourier transform infrared spectroscopy. The results were compared with the ones obtained from spin coated films. Poor chemical properties were observed for films deposited starting from chloroform solutions, possibly due to the formation of Cl radicals interacting with the polymer molecules. When using toluene as solvent, the spectra characteristics improved with the increase of the laser fluence. The best results were obtained using THF as solvent. To investigate the effect of the deposition parameters on the optical properties of the films, photoluminescence (PL) measurements were performed. The PL measurements confirmed the existence of defects in PFO films deposited using a chloroform solutions, evidencing the presence of their emission bands. The glassy phase was revealed in films deposited using toluene solutions. The THF seems to be the best solvent to preserve the PFO structure in MAPLE depositions. In conclusion, the MAPLE technique is effective in PFO thin film deposition, also avoiding oxidation problems. Further MAPLE film depositions are planned with the aim to enhance the film uniformity. Functionality tests are in progress to integrate the PFO films in electro-optic devices.

References

1. A. Piqué, R.A. McGill, D.B. Chrisey, D. Leonhardt, T.E. Mslna, B.J. Spargo, J.H. Callahan, R.W. Vachet, R. Chung, M.A. Bucaro, Thin Solid Films **355–356**, 536 (1999)
2. B.R. Ringeisen, J. Callahan, P.K. Wu, A. Piqué, B.J. Spargo, R.A. McGill, M. Bucaro, H. Kim, D.M. Bubb, D.B. Chrisey, Langmuir **17**(11), 3472 (2001)
3. M.A. Hernandez-Perez, C. Garapon, C. Champeaux, P. Shahgaldian, A. Coleman, J. Mugnier, Appl. Surf. Sci. **208**, 658 (2003)
4. J. Sagawa, S. Nagare, M. Senna, Appl. Surf. Sci. **244**, 611 (2005)
5. G. Shanmugam, P. Polavarapu, Biophys. Chem. **111**, 73 (2004)
6. U.K. Laemmli, Nature **227**, 680 (1970)
7. M.T. Bernius, M. Inbasekaran, J. O'Brien, W.S. Wu, Adv. Mater. (Weinheim, Ger.) **12**, 1737 (2000)
8. A.W. Grice, D.D.C. Bradley, M.T. Bernius, M. Inbasekaran, W.S. Wu, E.P. Woo, Appl. Phys. Lett. **73**, 629 (1998)
9. J.J.M. Halls, A.C. Arias, J.D. Mackenzie, W.S. Wu, M. Inbasekaran, E.P. Woo, R.H. Friend, Adv. Mater. (Weinheim, Ger.) **12**, 498 (2000)
10. H. Sirringhaus, R.J. Wilson, R.H. Friend, M. Inbasekaran, W. Wu, E.P. Woo, M. Grell, D.D.C. Bradley, Appl. Phys. Lett. **77**, 406 (2000)
11. D.M. Bubb, P.K. Wub, J.S. Horwitz, J.H. Callahan, M. Galicia, A. Vertes, R.A. McGill, E.J. Houser, B.R. Ringeisen, D.B. Chrisey, J. Appl. Phys. **91**(4), 2055 (2002)
12. J.P.R. Wells, D.G. Lidzey, P.J. Phillips, D.A. Carder, A.M. Fox, Appl. Phys. Lett. **85**, 3080 (2004)
13. W. Zhao, T. Cao, J.M. White, Adv. Funct. Mater. **14**(8), 783–790 (2004)
14. M. Sims, D.C. Bradley, M. Ariu, M. Koeberg, A. Asimakis, M. Grell, D.G. Lidzey, Adv. Funct. Mater. **14**, 765 (2004)
15. M. Ariu, M. Sims, M.D. Rahn, J. Hill, A.M. Fox, D.G. Lidzey, M. Oda, J. Cabanillas-Gonzales, D.C. Bradley, Phys. Rev. B **67**, 195333 (2003)
16. M.E. Caruso, S. Lattante, R. Cingolani, M. Anni, Appl. Phys. Lett. **88**, 181906 (2006)
17. T.T. Khan, P. Sreerunothai, L.M. Hert, M.J. Banach, A. Kohler, Phys. Rev. B **69**, 085201 (2004)