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1. Introduction

Polymer materials have been used for electronic, optical and bio micro/nano devices. Polymer device fabrication technologies based on replication methods including hot embossing (Becker & Heim, 2000; Park et al., 2003; Shinohara et al., 2007b), injection molding (Becker et al., 1986; Svedberg et al., 2003), ultraviolet (UV) imprinting (Haisma et al., 1996; Kawaguchi et al., 2007; Shinohara et al., 2008d) and casting (Duffy et al., 1998; Slentz et al., 2001) can reduce costs. Polymer bonding technologies have also been required for sealing or stacking the devices. Some examples of bonding methods have been reported, including thermal direct bonding (Spierings & Haisma, 1994; Chen et al., 2004; Shinohara et al., 2007b), solvent bonding (Wang et al., 2002; Lin et al., 2007), and bonding using other intermediate layer (Graß et al., 2001; Lei et al., 2004). Low-temperature bonding technologies are required with deformation of the previous surface structures as small as possible.

On the other hand, surface modification for biocompatibility is one of the most important processes for biochips. Polymer surface modification methods are classified into two categories. One is modification of the original surface (e.g., plasma treatment (Lianos et al., 1994; Kamińska et al., 2002; Chai et al., 2004; Lai et al., 2006), UV irradiation (Peeling & Clark, 1981; Murakami et al., 2003; Hozumi et al., 2004; Diaz-Quijada et al., 2007; Kim et al., 2009). The other is coating with other materials (Ratner, 1995; Oehr, 2003; Liu et al., 2004; Bi et al., 2006).

In this chapter, two low-temperature bonding technologies are described. Section 2 introduces low-temperature direct bonding methods of poly (methyl methacrylate) (PMMA) or cyclo-olefin polymer (COP), and their applications of microchannel devices. Section 3 describes surface hydrophilic treatment method using aromatic polyurea film, and bonding method using the polyurea film.

2. Low-temperature direct bonding of PMMA or COP

2.1 Surface pretreatment for low-temperature bonding

In our previous study, we developed a fabrication method for micro-scale flow devices by combining hot embossing and direct bonding techniques using a PMMA material. Direct bonding is superior to polymerize bonding or adhesive bonding because of its low optical

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loss in a bonded interface (Shinohara et al., 2007b). In this method, we fabricated flow channels around the glass transition temperature (T_g) of the material. Because of the applied pressure as well as heat during the direct bonding process, deformation of the channel was observed, although it was not a big problem in cell analysis. However, for single bio-molecule level analysis, which uses high-performance optical detection systems, high optical transparency of the material and nanometer-scale accuracy of the fabrication technologies are required.

In order to bond at lower than T_g , surface pretreatment was applied. Fig. 1 shows fabrication process of a polymer microchip using low-temperature direct bonding. First, silicon mold was fabricated by conventional photolithography and Deep-RIE (reactive ion etching) (Fig. 1 (a)). Microchannel patterns were formed by hot embossing (Fig. 1 (b)) (Shinohara et al., 2007b). After the microchannel plate and a lid were pretreated (Fig. 1 (c)), the microchannel was realized by the direct bonding (Fig. 1 (d)).



Fig. 1. Fabrication process of polymer microchip using low-temperature direct bonding (Shinohara et al., 2007a)

Examples of typical pretreatment methods are oxygen plasma, atmospheric-pressure oxygen plasma, UV/O_3 , and VUV (vacuum UV) $/O_3$. Typical treatment conditions of the equipments were shown in Table 1.

Oxygen plasma was generated in a plasma activated bonding system (EVG810LT from EV Group Co.). Oxygen plasma can be generated between parallel electrodes in the vacuum chamber. Since the radiofrequency (397 kHz) was lower than that of other conventional plasma treatment systems (13.56 MHz or higher), the damage on the surfaces was expected to be smaller. Atmospheric-pressure oxygen plasma was generated by plasma cleaning unit (Aiplasma from Panasonic Electric Works, Ltd.), using dielectric-barrier discharge (Sawada, 2003). In this equipment, high-density active plasma can be expelled from a nozzle supplying mixed gas (98 % Ar and 2 % O₂) under atmospheric pressure. After oxygen plasma irradiation, the molecular bonds (e.g. C-H) on the polymer surface are expected to be dissociated and incorporated oxygen radicals. Polar oxidized components were increased because of the incorporation (Lianos et al., 1994; Chai et al., 2004). This surface state is considered to enhance the bonding reaction at the interface.

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Condition	Oxygen plasma	Atmospheric plasma	UV/O ₃	VUV/O ₃
Gas	O ₂	Ar 98%, O ₂ 2%	O ₂	O ₂
Power (W)	200	80	-	-
UV wavelength (nm)	-		185, 254	172
Chamber pressure (p) (MPa)	8.0×10-5	0.1	0.1	5.0×10-2
Exposure time (<i>t</i>)	30 sec	0.6 sec	20 min	30 min

Table 1. Typical treatment conditions of oxygen plasma, atmospheric-pressure oxygen plasma, UV/O_3 , and VUV/O_3 (Shinohara et al., 2007a)



Fig. 2. Schematic diagram of VUV/O3 equipment (Shinohara et al., 2008b)

The UV/O₃ system (NL-UV253 from Nippon Laser & Electronics Lab.,) has three low-pressure UV lamps that radiate 185 nm and 254 nm lights in wavelength. In the presence of O₂, the 185nm UV is absorbed by O₂ to generate the atomic species in ground state O(³P). O(³P) can react with O₂ to form O₃. If this O₃ absorbs the 254-nm UV, excited oxygen atoms (O(¹D)) with 190 kJ/mol excitation energy are generated (Wang & Ray, 2000). The VUV/O3 system (UER20-172 from Ushio Inc.) has a dielectric barrier discharge excimer lamp filled with Xe gas and radiates light of a central wavelength of 172 nm (VUV). The VUV/O₃ system is shown in Fig. 2. Oxygen gas was introduced into the chamber after evacuation. The VUV generates not only O3 and O(1D) in the same manner as the 185-nm and 254-nm UV lights, but is also absorbed directly by O_2 in the chamber to generate O(1D) (Kaspar et al., 2003). The 172-nm UV light irradiance on the sample surface can be controlled by the oxygen pressure and the distance between the lamp window and the sample (d) (Hozumi et al., 2004; Shinohara et al., 2008b). In UV (VUV)/O₃ treatment, O(¹D) plays important roles on surface activation (Hozumi et al., 2004). Polar oxidized components were also increased as well as the oxygen plasma treatments (Peeling & Clark, 1981; Diaz-Quijada et al., 2007; Kim et al., 2009). Since absorption coefficient of O₂ at the 172-nm UV light are approximately 20 times greater than that at the 185-nm

(Watanabe et al., 1953), the efficiency of O(¹D) generated by VUV/O₃ treatment is better than that by UV/O₃. Thus, it is expected that the activation by the VUV/O₃ is more effective than that by UV/O₃. In addition, the UV light is expected to dissociate chemical bonds of polymer as C-C, C-O and C-H. Main or side chain cleavage of the polymer causes degradation of polymer so as to generate low- T_g layer on the surface (Truckenmüller et al., 2004). It is considered to be act as an adhesion layer for the direct bonding.

2.2 Bonding strength

Bonding strengths of PMMA plates (Acrylyte E IR from Mitsubishi Rayon Co., Ltd.) were measured by a tensile test method (Shinohara et al., 2007a). The results were shown in Fig. 3. In this figure, red broken lines indicate the values for direct bonding under temperature of 95 °C, pressure of 1.25 MPa and annealing time of 25 min, without any surface treatments. The bonding strengths were same or stronger than that bonded around T_g .

Bonding strengths of oxygen plasma-treated COP plates (Zeonex480 from Zeon Co.) measured by the tensile test were higher than 1 MPa. Bulk distraction was observed from the bonded sample after tensile test while no interface separation was observed. The bonding strengths of pretreated COP samples were also measured by razor blade method (Maszara et al., 1988). The bonding strength at room temperature was approximately 0.6 J/m². The strength was increased (~ 8 J/m²) after annealing at 70 °C (Mizuno et al., 2005a).



Fig. 3. Dependence of bonding strength of two PMMA plates on the annealing temperature (Shinohara et al., 2007a)

2.3 Shallow microchannel

A PMMA microchip which have fine channel of 5 μ m in depth and 150 μ m in width was fabricated by low-temperature direct bonding (bonding temperature of 75 °C) as shown in Fig. 4. (Shinohara et al., 2007a). The shallow microchannel was successfully fabricated without deformation, boids and leakages. To controlled conditions of surface treatment and bonding, the shallow microchannel can be also realized using COP materials (Shinohara et al., 2009b).

Fig. 5 shows a PMMA microchip which has two shallow dams of about 5 μ m gaps (Shinohara et al., 2006). The dam structures were kept after low-temperature bonding. The

flow behaviors of the dams were evaluated with fluorescent beads. Large microbeads (diameter: $5.7 \mu m$) were completely trapped and filled between two dams, while small microbeads (diameter: $1.0 \mu m$) were passed through the dams, as shown in Fig. 5 (c).



Fig. 4. A shallow PMMA microchip: (a) whole and (b) magnified view; (c) cross-section of a shallow microchannel (width: 150 μ m, depth: 5 μ m) (Shinohara et al., 2007a)



Fig. 5. A PMMA microchip which has two shallow dams of about 5 μ m gaps: (a) design; (b) whole view and optical micrograph near a dam; (c) flow behaviour near a dam (Shinohara et al., 2006)

2.4 MCE-ESI-MS microchip

Mass spectrometry (MS) is one of the useful detection methods for microchip electrophoresis (MCE). The advantages of combining MCE and MS (MCE-MS) include high sensitivity, no need for the derivatization of samples and valuable for the analysis of complex mixtures such as biomedical samples. In many cases, the electrospray ionization (ESI) method is used as an interface of MCE-MS (MCE-ESI-MS). Tapered capillary of a spray nozzle was generally connected directly to the channel outlet (Li et al., 2000; Zhang et al, 2001, Tachibana et al., 2003; Tachibana et al., 2004). However, there are a few technical problems caused by the dead volume at a connecting joint between the spray nozzle and the microchip. Efficiency of the spray is strongly depends on the structure of the nozzle.



Fig. 6. A MCE-ESI-MS microchip made of two COP plates: (a) design; (b) SEM micrograph of the electrospray tip; MS spectra of (c) arginine and (d) caffeine (Shinohara et al., 2008a)

We developed a MCE-ESI-MS microchip made of two COP plates as shown in Fig. 6 (Shinohara et al., 2008a). An ESI emitter tip was fabricated directly on the opening of a separation channel by machining and electron beam evaporation of Au. Since the direct bonding is performed at the temperature lower than T_g , deformation of the channel structure was negligible. There was no crack at the bonded interface even after structuring the tip because of its sufficient bonding strength. Since the structure of the nano-electrospray tip enables neglected dead volume in the ESI interface, an efficient spray of a

sample solution and higher separation efficiency are expected. The success rate of Taylor cone generation was increased with decreasing the tip angle (α). Arginine and caffeine were successfully separated and detected as [M+H]⁺ in the MCE-ESI-MS analysis at α = 30 °, the separation voltage for MCE of 1.3 kV, and the ESI voltage (potential difference between the nano-electrospray tip and the MS orifice) of 2.0 kV, as shown in Fig. 6 (c) and Fig. 6 (d).



Fig. 7. Results of stability and reproducibility test: (a) reproducibility of the peak height detected as MS spectrum; (b) photomicrographs of the nano-electrospray tip after 1st, 5th, 10th, and 14th run (Shinohara et al., 2008c)

For stability and reproducibility test, MCE-ESI-MS analysis was carried out repeatedly, by using caffeine in 10 mM ammonium acetate as a sample solution (Shinohara et al., 2008c). A MCE-ESI-MS microchip was reused and the reproducibility of the peak heights detected as MS spectrum was observed. Fig. 7 (a) shows the peak heights at 1st, 3rd, 5th, 7th, 10th, 12th, and 14th run. Stable MS detection was achieved and reproducible peak heights were kept up to 13 times. The residual standard deviation (RSD) of the peak height was 9.4 %. At the 14th run, the peak was not detected. Fig. 7 (b) shows photomicrographs of the nanoelectrospray tip after 1st, 5th, 10th, and 14th run. After 10th run, optical transparency of the tip was increased obviously. It is indicated that thickness of the Au film decreased. After 14th run, the decrease area was expanded, and deformation of the tip structure was observed. The obvious decrement of the peak at 14th run was caused by the deformation or damage of the Au electrode. The damages of the bonding interface were not observed. The Au thickness looked thinner; however, it was still remained on the COP tip. These results indicate that bonding strength of the COP plates and the adhesion strength of the Au film are strong enough. The stability and reproducibility of the fabricated nanospray tip is sufficient in practical use.

3. Low-temperature polymer bonding using polyurea film

3.1 Hydrophilic treatment of polyurea film using VUV/O3

In our previous work, we fabricated and evaluated a blood analysis chip made of PMMA (Mizuno et al., 2005b; Shinohara et al., 2005). This chip has microchannel array, which equivalent diameter is 6 μ m. When human whole blood is flowed into the microchannels, platelet aggregation was observed after channel passage due to activation of platelet. This

chip is used for the evaluations of the shear stress sensitivity of platelets, the adhesion of white blood cells and the hardness of red blood cells from blood transit time as well as the blood flow images (Kikuchi et al., 1992; Kikuchi et al., 1994). Hydrophilic treatment on the microchannels was required to flow the blood smoothly and not to adhesion of biomaterials. Direct hydrophilic treatment in section 2 was not sufficient because of low stability or low hydrophilicity on the treated surface (see Fig. 16). In this case, aromatic polyurea film coating was selected because of the advantages in visible transparency, non-toxicity, high purity and uniform film thickness (Shinohara et al., 2005). The aromatic polyurea film was prepared by vapor deposition polymerization of 4,4'-diaminodiphenyl methane (MDA) and 4,4'-diphenylmethane diisocyanate (MDI) (Takahashi et al., 1989) as shown in Fig. 8. After coating, highly hydrophilic surface was realized by annealing (50 - 150 °C) and exposing for O₃ at the same time under atmospheric pressure. This treated film had highly hydrophilic surface, water contact angle was smaller than 30 °, and hydrophilic surface was kept for long time (longer than a month) (Shinohara et al., 2005). However, the annealing process for hydrophilic treatment causes bending of the PMMA chip. On the other hand, the film surface was recovered to hydrophobic after washing by water. For reproducible measurements, improvement of the surface stability is required.

We improved the hydrophilic treatment of polyurea and removed the annealing process using VUV/O₃. The VUV/O₃ system used in section 2 was also used (see also Fig. 2). The polyurea surface is treated by the generated gases (O₃ and O(¹D)). Then, direct irradiation effect of the VUV light for surface modification is expected to be small in case of large *d*. The light intensity at the sample surface decreases because the VUV is absorbed by oxygen gas in the chamber. Therefore, O₃ and O(¹D) are only generated near the lamp window, and these gases are spreaded over the chamber by diffusion. Since this treatment is carried out at room temperature, the deformation of the sample structure is negligible.



Fig. 8. Reaction scheme of aromatic polyurea

To evaluate the surface treatment effect, transit time of water contact angle after VUV/O₃ was measured under several conditions, as shown in Fig. 9 (Shinohara et al., 2008b). The untreated polyurea film has low hydrophilic surface, contact angle of about 80 °, while the treated films keep contact angles smaller than 45 ° for long time. Especially under the condition of chamber pressure (*p*) of 3.0 x 10⁴ Pa, and exposure time (*t*) of 20 min, contact angle smaller than 20 ° was realized and kept about two months. Even after very hard condition of ultrasonic cleaning in de-ionized water for 3 min, contact angle of smaller than 40 ° was realized with the VUV/O₃-treated sample (Shinohara et al., 2008b). These results indicate that the VUV/O₃-treated polyurea was improved surface stability even after

washing by water. In addition, the contact angle decreases with increasing the *d*, as shown in Fig. 10 (Shinohara et al., 2008b). Since the VUV light intensity decreases with distance from the light source, the direct irradiation effect of the VUV light (e.g., cross-linking (Sato et al., 1994), breakage of main polyurea structure) expected to be avoided.



Fig. 9. Transit time of water contact angle on polyurea surface after VUV/O₃ treatment (d = 142 mm) (Shinohara et al., 2008b)



Fig. 10. Contact angle of de-ionized water versus distance between the lamp window and the sample ($p = 3.0 \times 10^4$ Pa, t = 20 min) (Shinohara et al., 2008b)

The polyurea film was applied for PMMA blood analysis chip. As in the case of a conventional silicon chip (Kikuchi et al., 1992; Kikuchi et al., 1994), polyurea-coated PMMA chip was contacted with flat glass plate mechanically. The performance of the surface treatment was evaluated by actual human whole blood flow. The adhesion of platelets and white blood cells was significant in the case of a thermal-oxydized silicon chip (Fig. 11 (a)), while the PMMA chip coated polyurea film can reduce the adhesion of platelets and white

blood cells (Fig. 11 (b)), even after ultrasonic cleaning in surfactant induced water (Fig. 11 (c)) (Shinohara et al., 2008b).



Fig. 11. Images of blood flow: (a) conventional chip made of Si for reference; (b) PMMA chip coated polyurea film; (c) reused PMMA chip after ultrasonic cleaning with surfactant-induced water (Shinohara et al., 2008b)

3.2 Thermal bonding using hydrophilic polyurea film

The hydrophilic polyurea film was used as intermediate bonding layers (Shinohara et al., 2009a). Fig. 12 shows a fabrication process of a microchip which has highly-hydrophilic microchannels. The polyurea was coated on the channel plate and the lid by vapor deposition polymerization (Fig. 12 (a)). Next, the polyurea-coated plates were treated with VUV/O₃ (Fig. 12 (b)). After VUV/O₃ treatment, the plates were brought into contact and then pressed (Fig. 12 (c)). The typical bonding temperature was 85 °C, and the pressure was 3 MPa for 20 min in the case of PMMA plates (Comoglass from Kuraray Co., Ltd.). Fig. 13 (a) and (b) shows a prototype PMMA microchip. Void-free structure was realized over the whole sample surface. Since the bonding temperature is lower than the Tg of the PMMA, negligible deformation of the channel structure is obtained. To observe its flow behavior, a 5- μ L methylene blue aqueous solution droplet was applied onto a port (as indicated black arrow in Fig. 13 (a)) on the fabricated microchip (Shinohara et al., 2009a). Its flow behavior at the cross-junction is shown in Fig. 13 (c). All the microchannels were filled by capillary force. There was no leakage or obstacles to smooth fluidic flow at the bonded interface.

To evaluate the surface modification and annealing effect, contact angles of water (H₂O), glycerin (C₃H₅(OH)₃), formamide (HCONH₂) and diiodomethane (CH₂I₂) on the polyurea surface were measured (Shinohara et al., 2009a). The results were shown in Fig. 14. After the VUV/O₃ treatment, contact angles of water, glycerin, and formamide decreased dramatically, and the contact angles were kept even after annealing of 85 °C for 20 min. This result indicates that the highly hydrophilic surface of the microchannel was also realized after the above-mentioned bonding process.

In addition, surface free energy (γ_s), its polar ($\gamma_s p$) and dispersive ($\gamma_s d$) components ($\gamma_s = \gamma_s p + \gamma_s d$) were calculated using these contact angle results, according to Owens-Wendt theory (Owens & Wendt, 1969). The results were shown in Fig. 15 (Shinohara et al., 2009a). After VUV/O₃ treatment, the $\gamma_s p$ was increased significantly, while the $\gamma_s d$ was decreased. The result indicated that the additional new polar groups (e.g., OH, C=O, COOH) were created after the treatment. After annealing, the $\gamma_s p$ was decreased while the $\gamma_s d$ was increased. These results indicate two possibilities. One is that conformational transformations of the

generated polar groups occurred. The other is that unreacted polymer tails (NH₂ or N=C=O) of polyurea were consumed by further polymerization during the annealing. In Fig. 8, the as-deposited polyurea film of only about five monomers (n = 5) is formed at room temperature (Wang et al., 1993). Further polymerization takes place (n > 5) when as-deposited films are annealed (without any surface treatment) by consuming the unreacted



Fig. 12. Fabrication of a microchip which has highly-hydrophilic microchannels: (a) polyurea coating; (b) VUV/O_3 treatment; (c) thermal bonding (Shinohara et al., 2009a)



Fig. 13. Prototype PMMA microchip using polyurea film: (a) design; (b) whole view; (c) observation of flow behavior at the cross-junction (Shinohara et al., 2009a)



Fig. 14. Contact angles of water, glycerin, formamide, and diiodomethane on the polyurea surface before and after VUV/O₃ treatment ($p = 3.0 \times 10^4$ Pa, t = 20 min, d = 142 mm) (Shinohara et al., 2009a)



Fig. 15. Surface free energies of polyurea before and after VUV/O_3 treatment (Shinohara et al., 2009a)

polymer tails to form amid bonds (Takahashi et al., 1991). These transformations or polymerization could also have occurred at the interface of the two polyurea films during the bonding process.

To compare hydrophobic recovery with other low-temperature direct bonding, the water contact angle on the polyurea, the COP, and the PMMA surface before and after surface treatment, and after the treatment and annealing (at 85 °C for 20 min) were measured (Shinohara et al., 2009a). Oxygen plasma was selected for surface treatments of COP and PMMA. The results were shown in Fig. 16. In the case of the COP, a highly hydrophilic surface (~20 °) was realized after oxygen plasma treatment. However, the hydrophilic surface was not maintained after the annealing. In the case of the PMMA, the treatment effect was weak. From these results, the bonding using the polyurea as the intermediate layer is the best method from the hydrophilicity viewpoint.



Fig. 16. Water contact angle in three conditions (untreated, after treatment, after treatment and annealing) on VUV/O₃-treated polyurea, oxygen plasma-treated COP (100 W, $p = 4.0 \times 10^{-5}$ MPa, t = 30 sec), and oxygen plasma-treated PMMA (200 W, $p = 0.8 \times 10^{-5}$ MPa, t = 30 sec) (Shinohara et al., 2009a)

4. Conclusion

In this chapter, two low-temperature bonding technologies, direct bonding of PMMA or COP, and bonding using surface hydrophilic polyurea film were described. The bonding was carried out at temperature lower than T_g of the polymer plates.

The low-temperature direct bonding was realized by surface pretreatment such as oxygen plasma, atmospheric-pressure oxygen plasma, UV/O_3 , and VUV/O_3 . Reasonable bonding strength was realized with negligible deformation. Shallow microchannels of about 5 mm gaps were successfully fabricated. By using this bonding technology, a MCE-ESI-MS microchip was developed. Arginine and caffeine were successfully separated and detected as [M+H]⁺ in the MCE-ESI-MS analysis.

On the other hand, a novel hydrophilic treatment method in microchannel surface using aromatic polyurea was developed. The polyurea was changed highly hydrophilic (water contact angle < 20 °) after VUV/O₃ treatment, and the treated film kept highly hydrophilic surface for long time (~ 2 months). The polyurea film was applied for PMMA human blood analysis chip. The new chip can reduce the adhesion of platelets and white blood cells. The technology of the surface hydrophilic treatment of polyurea can be applied to low-temperature bonding. The VUV/O₃-treated polyurea film was used as intermediate bonding layers. The highly hydrophilic surface of the microchannel was retained after the thermal bonding process. There was no leakage or obstacles to smooth fluidic flow at the bonded interface. For actual micro-biochip fabrication with this method, the post-hydrophilic treatment after bonding process is expected unnecessary.

We are currently investigating these bonding mechanisms and optimizing these pretreatment conditions. In addition, these bonding methods will be applied to other polymer microchips.

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The evolution of solid-state circuit technology has a long history within a relatively short period of time. This technology has lead to the modern information society that connects us and tools, a large market, and many types of products and applications. The solid-state circuit technology continuously evolves via breakthroughs and improvements every year. This book is devoted to review and present novel approaches for some of the main issues involved in this exciting and vigorous technology. The book is composed of 22 chapters, written by authors coming from 30 different institutions located in 12 different countries throughout the Americas, Asia and Europe. Thus, reflecting the wide international contribution to the book. The broad range of subjects presented in the book offers a general overview of the main issues in modern solid-state circuit technology. Furthermore, the book offers an in depth analysis on specific subjects for specialists. We believe the book is of great scientific and educational value for many readers. I am profoundly indebted to the support provided by all of those involved in the work. First and foremost I would like to acknowledge and thank the authors who worked hard and generously agreed to share their results and knowledge. Second I would like to express my gratitude to the Intech team that invited me to edit the book and give me their full support and a fruitful experience while working together to combine this book.

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