Surface Functionalization

International Edition: DOI: 10.1002/anie.201509937
German Edition: DOI: 10.1002/ange.201509937

Simultaneous Dual Encoding of Three-Dimensional Structures by Light-Induced Modular Ligation

Tanja K. Claus, Benjamin Richter, Vincent Hahn, Alexander Welle, Sven Kayser, Martin Wegener, Martin Bastmeyer, Guillaume Delaittre,* and Christopher Barner-Kowollik**

Abstract: *A highly efficient strategy for the simultaneous dual surface encoding of 2D and 3D microscaffolds is reported. The combination of an oligo(ethylene glycol)-based network with two novel and readily synthesized monomers with photoreactive side chains yields two new photoresists, which can be used for the fabrication of microstructures (by two-photon polymerization) that exhibit a dual-photoreactive surface. By combining both functional photoresists into one scaffold, a dual functionalization pattern can be obtained by a single irradiation step in the presence of adequate reaction partners based on a self-sorting mechanism. The versatility of the approach is shown by the dual patterning of halogenated and fluorescent markers as well as proteins. Furthermore, we introduce a new ToF–SIMS mode ("delayed extraction") for the characterization of the obtained microstructures that combines high mass resolution with improved lateral resolution.*

Recently, well-defined two- and three-dimensional (2D and 3D) micro- or nanostructures have received increasing attention for applications in numerous fields, such as nanophotonics,[1] microelectronics,[2] and cell biology.[3] Therefore, diverse techniques to achieve nano- or micropatterning in two or three dimensions have been developed and improved over the last decade (e.g., laser-induced chemical vapor deposition,[4] microcontact printing,[5] electron- or focused ion-beam lithography,[6] soft lithography,[7] and photolithography),[8] with direct laser writing (DLW)[9] being one of the most versatile techniques. DLW, which is based on the two-photon polymerization of multifunctional (meth)acrylates, has proven to be a powerful method for the fabrication of sophisticated devices, such as photonic crystals,[10] optical waveguides,[11] lab-on-a-chip systems,[12] or cell-guiding scaffolds.[13] Thus, substantial work has been carried out for improving writing resolution[14] as well as writing speed.[15] However, only little efforts were directed towards increasing the chemical diversity or the surface functionalization of the written scaffolds. One method for the post-modification of surfaces or 3D structures entails the coating with substances bearing reactive moieties that can be addressed by an efficient ligation method. Our group has established various two-step surface-functionalization methods that are based on the use of silane or polydopamine anchoring groups in combination with effective light-triggered transformations, enabling the formation of well-defined surface patterns with spatiotemporal control.[16] Furthermore, some success was achieved by employing the light-triggered Diels–Alder reaction of *ortho*-quinodimethanes onto DLW scaffolds, generating spatially controlled protein patterns in a very precise fashion.[17] However, this method is time-consuming owing to several post-modification steps, and the activation of the photoreaction requires a custom-built laser setup, as opposed to the commercial Nanoscribe setup that we employ here (see the Supporting Information).

An alternative technique for the post-modification of DLW-based scaffolds is to change the components involved in the scaffold formation itself to render it chemically addressable after fabrication.[18] In one approach, the scaffold was established by employing a multifunctional thiol and a vinyl ether linker for thiol–ene polymerization. As a result, the

[*] T. K. Claus, Dr. A. Welle, Dr. G. Delaittre, Prof. Dr. C. Barner-Kowollik
Preparative Macromolecular Chemistry
Institut für Technische Chemie und Polymerchemie
Karlsruhe Institute of Technology (KIT)
Engesserstrasse 18, 76131 Karlsruhe (Germany)
E-mail: alexander.welle@kit.edu
guillaume.delaittre@kit.edu
christopher.barner-kowollik@kit.edu
Homepage: http://www.macroarc.de

T. K. Claus, Dr. A. Welle, Prof. Dr. C. Barner-Kowollik
Institut für Biologische Grenzflächen (IBG)
Karlsruhe Institute of Technology (KIT)
Hermann-von-Helmholtz-Platz 1, 76344 Eggenstein-Leopoldshafen (Germany)

Dr. B. Richter, Prof. Dr. M. Bastmeyer
Cell- and Neurobiology, Zoological Institute
Karlsruhe Institute of Technology (KIT)
Haid-und-Neu-Strasse 9, 76131 Karlsruhe (Germany)

S. Kayser
ION-TOF GmbH
Heisenbergstrasse 15, 48149 Münster (Germany)

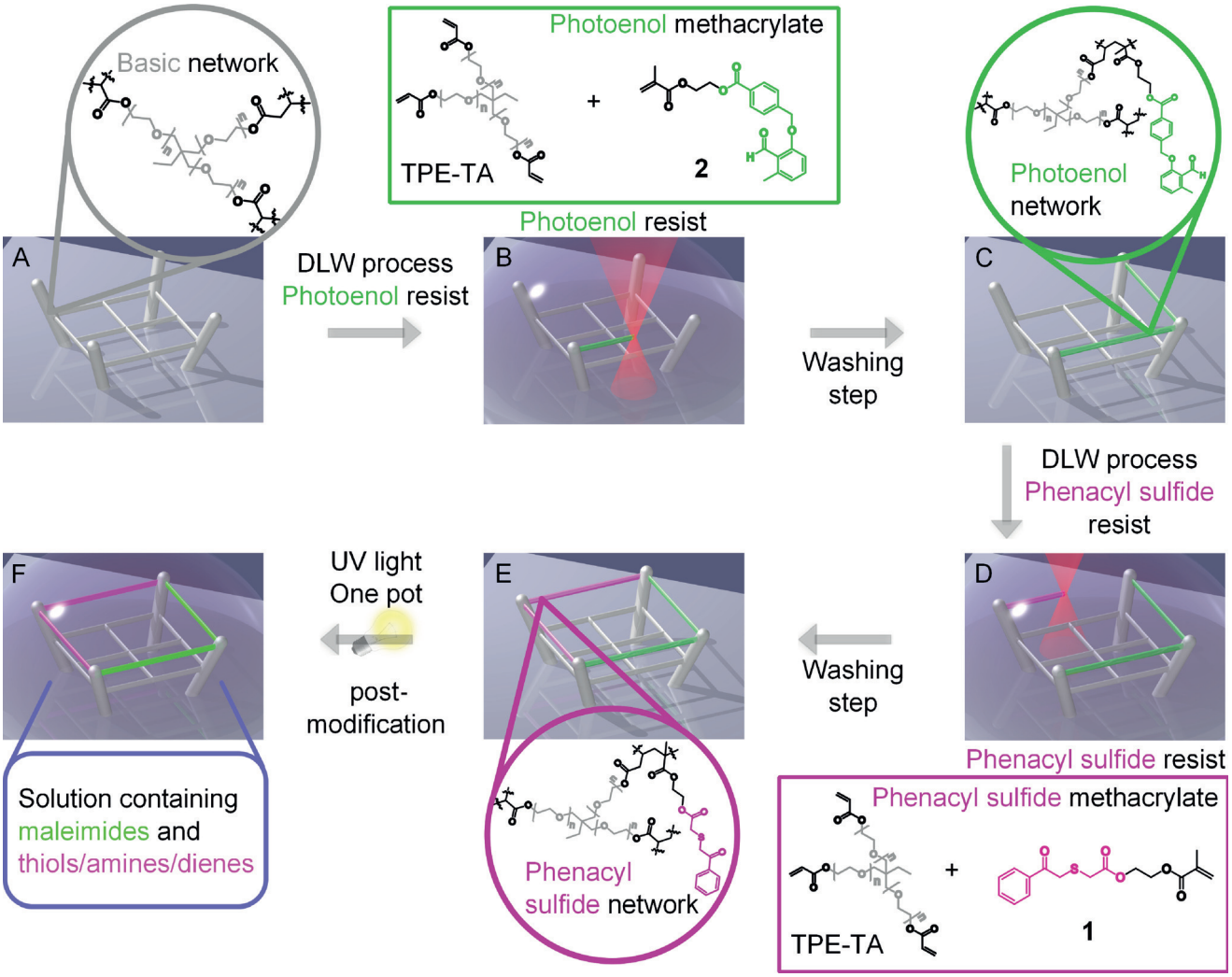
V. Hahn, Prof. Dr. M. Wegener
Institute of Applied Physics and Institute of Nanotechnology
Karlsruhe Institute of Technology (KIT)
Wolfgang-Gaede-Strasse 1, 76131 Karlsruhe (Germany)

Prof. Dr. M. Bastmeyer
Institut für Funktionelle Grenzflächen (IFG)
Karlsruhe Institute of Technology (KIT)
Hermann-von-Helmholtz-Platz 1, 76344 Eggenstein-Leopoldshafen (Germany)

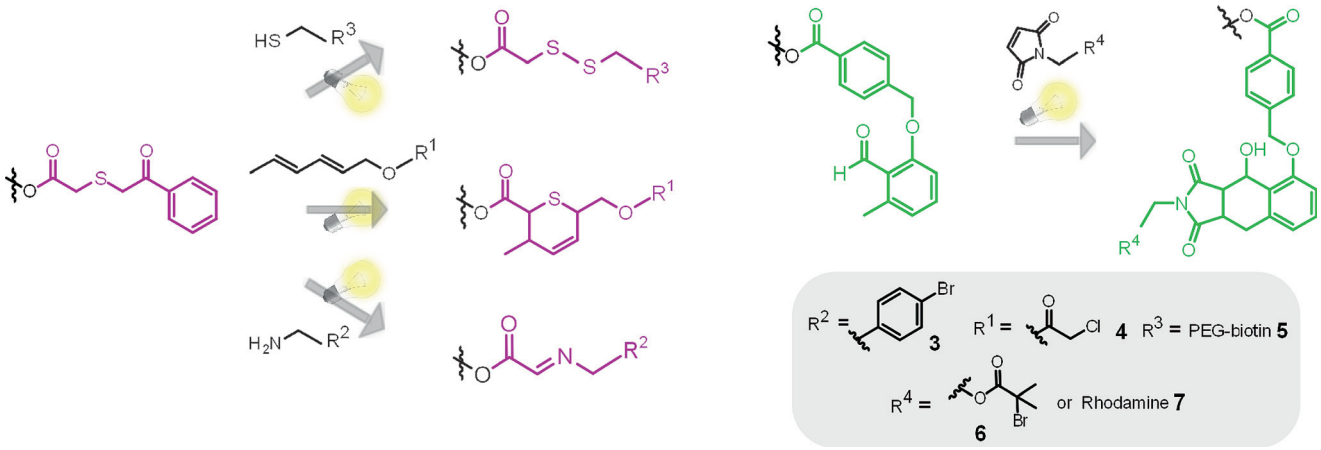
Dr. G. Delaittre
Institute for Toxicology and Genetics (ITG)
Karlsruhe Institute of Technology (KIT)
Hermann-von-Helmholtz-Platz 1, 76344 Eggenstein-Leopoldshafen (Germany)

Supporting information for this article can be found under http://dx.doi.org/10.1002/anie.201509937.

written networks exhibit residual thiol moieties at the surface and can be readily functionalized with maleimide derivatives.[18a] Unfortunately, this method lacks spatial control and also requires a custom-built laser setup. To overcome the aforementioned drawbacks, we have now developed novel photoresists that fulfill the following requirements: 1) The network should be based on (meth)acrylate monomers to enable its application in commercial DLW setups, 2) these monomers should contain reactive groups that can survive the writing process, and 3) these reactive groups should be addressable through orthogonal pathways to allow for spatial resolution. Indeed, by combining at least two reactive photoresists in the same microstructure and adequately selecting the reactive moieties, we achieved the multiple and simultaneous orthogonal functionalization of 3D microstructures for the first time. The current study thus goes beyond 2D patterning approaches based on orthogonal mechanisms.[19] To simplify the system, two new reactive monomers were mixed with a so-called basic resist consisting of commercially available components: a trifunctional acrylate as the network-forming component (trimethylolpropane ethoxylate triacrylate, TPE-TA) and a photoinitiator (Irgacure 369; Scheme 1). These mixtures were converted into microstructures displaying reactive groups, thereby making the previously employed post-DLW coating steps obsolete. As reactive moieties, we turned towards photoreactive groups. Two well-known motifs were chosen: phenacyl sulfide[20] and photoenol.[21] After photolysis, the former yields thioaldehydes, which can react with nucleophiles, such as amines, hydroxylamines, thiols, and nitrile oxides,[22] or in a cycloaddition with an appropriate



Scheme 1. Overview of the sample preparation process and the chemistry of the functional resists. A) Structure made with the basic resist consisting of pure TPE-TA monomer, which was polymerized in a first DLW process (not shown). B, C) Second DLW process with the first functional resist (here the photoenol resist, leading to a photoenol-containing network) building up another part of the microstructure. D, E) Third DLW process with the second functional resist (here the phenacyl sulfide resist) building up a third part of the microstructure (here consisting of a phenacyl sulfide containing network). F) Post-modification of the resulting microstructure consisting of three networks. A droplet of a solution containing functionalized maleimide and thiol-, amine-, or diene-functionalized molecules is added to the structure, and the sample is globally irradiated with UV light to induce the surface functionalization. The Irgacure 369 photoinitiator, present in the writing steps, was omitted for simplification.



Scheme 2. Summary of the photochemistry applied for the post-modification of the 2D and 3D scaffolds. Left: The phenacyl sulfide moiety reacting with either a thiol, a diene, or an amine in a UV-induced reaction. Right: The photoenol moiety reacting with maleimides upon UV irradiation.

diene.[23] Under UV irradiation, photoenols are in equilibrium with a diene species, which are also amenable to Diels–Alder cycloaddition. These two motifs were selected as they fulfill important requirements: 1) compatibility with the commercially available DLW setup (i.e., they remain intact during the writing process), 2) high reactivity in subsequent phototriggered reactions, and 3) a certain degree of chemical orthogonality[24] (i.e., they render simultaneous dual post-modification possible).

The new monomers are depicted in Scheme 1. By mixing phenacyl sulfide methacrylate (**1**) with the basic resist, the so-called phenacyl sulfide (PS) resist was obtained. In analogy, the photoenol (PE) resist consists of the photoenol methacrylate (**2**) and the basic resist. The basic resist was also employed to generate structures for control purposes. For the current study, we selected checkerboard- and boxing-ring-type structures to demonstrate our strategy in 2D and 3D, respectively. The overall sample preparation process for boxing rings is shown in Scheme 1.

Initially, the base of the structure is written onto a glass cover slip with the basic resist in a DLW process. To ensure the covalent attachment of the scaffold to the cover slip, the latter is silanized with 3-(trimethoxysilyl)propyl methacrylate. After developing (removal of non-polymerized starting material), the basic structure is obtained (Scheme 1 A). The functional photoresists are employed in a similar manner in two successive DLW processes to form additional arbitrary parts of the structure, here the upper-row beams (Scheme 1 B–E). The sequence order (photoenol resist first, then phenacyl sulfide resist or vice versa) can be chosen arbitrarily. Satisfactorily, despite the incorporation of a monofunctional monomer, which reduces the crosslinking density, it was clearly possible to produce small features with sufficient mechanical stability. Scanning electron microscopy (SEM) images of the obtained structures are depicted in Figure 1, and confirm that well-defined microstructures with homogeneous surface topography can be obtained. We then proceeded to examine the photoreactivity of the microstructures. A droplet of a reaction mixture containing the appropriate

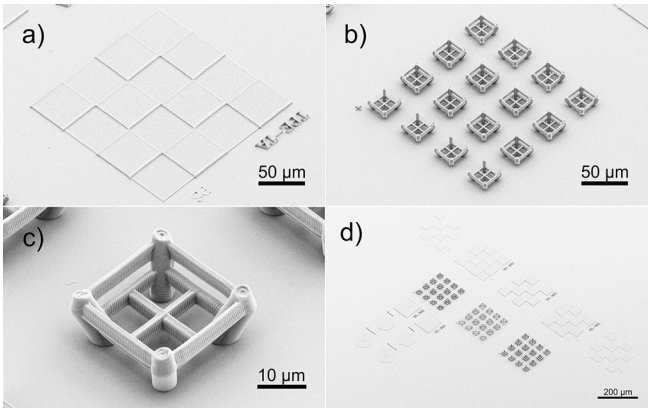


Figure 1. SEM images of the generated DLW structures. a) Checkerboard consisting of two resists (TPE-TA and PS). b) Overview of a grid of boxing-ring-like 3D scaffolds. c) Detailed image of one of the boxing rings. d) Overview of a complete sample with checkerboards and boxing-ring grids.

reaction partners (for details on the photoreactions, see Scheme 2) was added to the finished structures, and the cover slips were placed underneath a simple broadband UV lamp for 30 min (see Scheme 1 F).

The resulting functionalized scaffolds were subsequently subjected to time-of-flight secondary-ion mass spectrometry (ToF–SIMS) measurements to assess the success of the reaction as well as to obtain spatial information. To achieve both high spatial resolution and sufficient mass resolution, a novel ToF–SIMS imaging mode, the delayed extraction mode, was employed. This mode allows for a mass resolution of $m/\Delta m$ between 4000–5000 and a lateral resolution of 150 nm (for detailed information on the new ToF–SIMS mode, see the Supporting Information). For facile imaging, halogenated reaction partners were chosen (see Scheme 2). As the reaction partners for the thioaldehydes arising from the UV-induced decomposition of the phenacyl sulfide, a brominated amine (**3**; for nucleophilic addition) and

a chlorinated diene (**4**; for hetero-Diels–Alder cycloaddition) were selected to demonstrate its versatility with respect to specific reaction pathways. Furthermore, a brominated maleimide (**6**) was chosen as the dienophile reaction partner for the diene species generated from the photoenol upon irradiation. As mentioned above, employing a diene and a dienophile as reaction partners for the phenacyl sulfide and the photoenol, respectively, enables simultaneous dual patterning (see below).

The ToF–SIMS results for light-induced surface reactions in 2D (checkerboards) as well as in 3D (boxing rings) are summarized in Figure 2. The sums of the characteristic fragments are depicted in the colored overlay images (for the individual ion maps, see the Supporting Information). We first investigated structures containing only one type of photoreactive resist (a–f) before combining the two novel photoresists within the same structure (g,h).

The first two columns (Figure 2a–d) show the results for structures based on the functional phenacyl sulfide resist in combination with the basic, non-reactive resist using either brominated amine **3** (Figure 2a,b) or chlorinated diene **4** (Figure 2c,d). Aside from the green patches, which correspond to PEG (green channel, C_2HO), well-defined orange patches, which correspond to the localized grafting of brominated or chlorinated molecules (red channel), are clearly visible for both the 2D and 3D structures on a glass support (blue channel, SiO_2 and SiO_3H).

The ion maps in the third column (Figure 2e,f) show the results for the photoenol resist in combination with the basic, non-reactive resist. In this case, brominated maleimide **6** was employed as the reaction partner, and UV irradiation yielded a bromine pattern in 2D (Br yellow, PEG red) as well as in 3D (Br bright green). In all cases, no halogen signals could be detected in areas originally devoid of photoreactive moieties (see the individual ion maps in Figures S9 and S10).

Eventually, microstructures that combine the features of both the phenacyl sulfide and photoenol photoresists were employed for dual patterning by two simultaneous Diels–Alder cycloadditions (Figure 2g,h). In a one-pot reaction with chlorinated diene **3** and brominated maleimide **6**, a dual pattern of chlorine (red) and bromine (green) was obtained. Aside from the successful implementation of our strategy, the capability of the novel delayed-extraction ToF–SIMS mode for the imaging of fine chemical patterns in three dimensions is noteworthy. It is also interesting to note that the signals of specific marker fragments (e.g., bromine) decay very quickly upon primary ion beam exposure during the SIMS analysis of a given position of the scaffolds, leading to the conclusion that post-functionalization of the scaffolds occurs only at the surface (in a nanometer-thick layer) and not within the network formed during DLW.

To further investigate the potential of our system, we turned to dye and protein immobilization and assessed the outcome by confocal fluorescence microscopy. For this

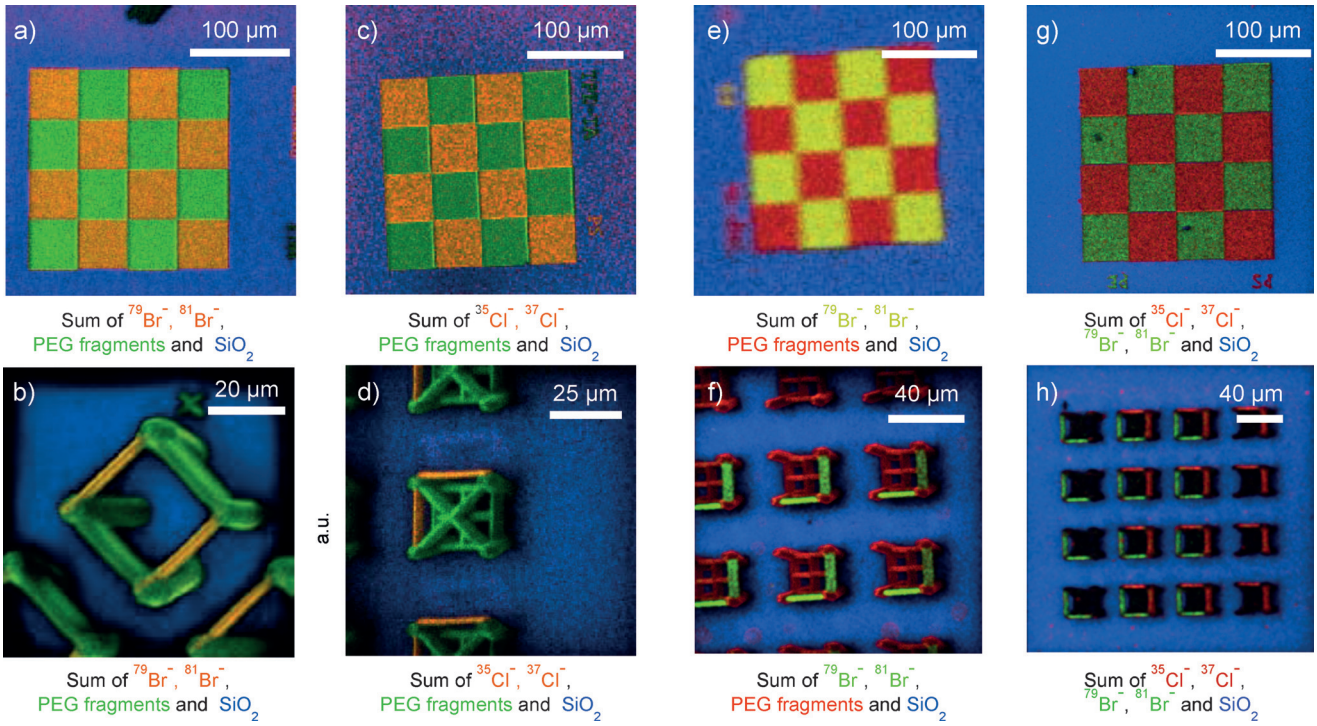


Figure 2. ToF–SIMS ion maps obtained after phototriggered functionalization of DLW structures with halogenated markers. The top row depicts checkerboard structures consisting of two distinct resists, and the bottom row shows 3D boxing-ring-like structures. All structures consist of the basic resist and one or two functional resists. a,b) Phenacyl sulfide resist and basic resist irradiated in the presence of brominated amine **3**. c,d) Phenacyl sulfide resist and basic resist irradiated in the presence of chlorinated diene **4**. e,f) Photoenol resist and basic resist irradiated in the presence of brominated maleimide **6** (image in (e) recorded in bunch mode, see the Supporting Information). g,h) Phenacyl sulfide resist and photoenol resist irradiated in the presence of both **4** and **6**.

purpose, a rhodamine maleimide derivative (Mal–Rhod, **7**) was chosen as a reaction partner for the photoenol resist based patches. A thiolated PEG–biotin derivative (biotin–PEG–SH, **5**) was employed for functionalization of the phenacyl sulfide resist based areas through formation of a disulfide linkage (see Scheme 2). Biotinylated areas can subsequently be stained with a fluorescent streptavidin conjugate (SAv-647; see the Supporting Information). The resulting fluorescence images are depicted in Figure 3. Similar

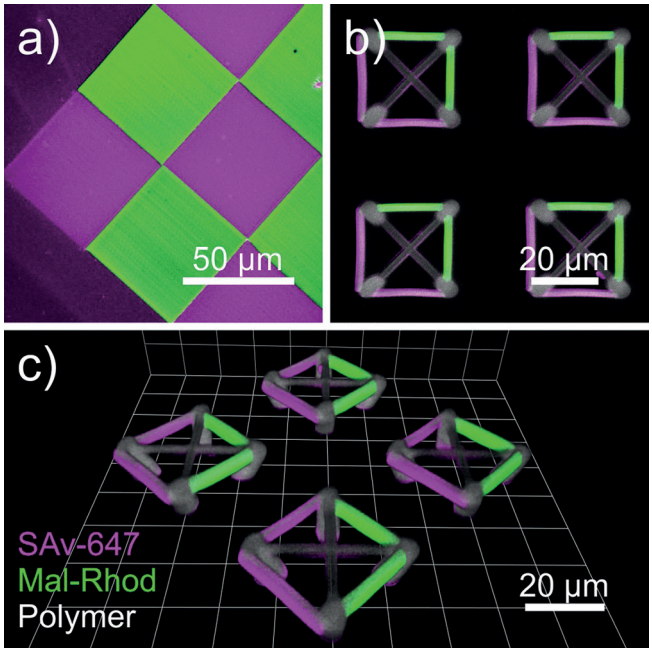


Figure 3. Confocal fluorescence microscopy images of dual-functionalized samples. The PS resist was functionalized with biotin–PEG–SH and subsequently stained with SAv-647 (pink). The PE resist was functionalized with Mal–Rhod (green). Gray: Autofluorescence of the basic resist (polymer). a) Merged image of the 2D checkerboard structure. b) Merged image of 3D boxing-ring-like structures. c) 3D reconstruction of confocal image stacks of the dual-functionalized 3D boxing rings. See Figure S15 for single-channel images.

to the ToF–SIMS images, clear fluorescence patterns were observed for the planar checkerboard structures (Figure 3a) as well as for the upper photoreactive beams of the 3D boxing-ring-like scaffolds (Figure 3b), indicating the success of the simultaneous dual light-induced patterning process. To obtain an even more convincing view of the three-dimensional character of the functionalization, a 3D reconstruction of the confocal image stacks of the dual-functionalized boxing rings is shown in Figure 3c. In a control experiment (see the Supporting Information), all steps were performed as for the sample shown in Figure 3, only leaving out the irradiation step. The results are shown in Figure S16. Only fluorescence arising from the SAv-647 present on the glass surface is detected, which additionally demonstrates the protein-repellent character of the novel photoresists, a beneficial property for applications in the field of protein patterning.

In summary, we have demonstrated the DLW fabrication of sophisticated 2D and 3D microscaffolds that consist of patches with distinct photoreactivity. This process is based on the use of two novel photoresists with photoreactive groups whose reactivity is orthogonal to the two-photon-induced polymerization process. These photoresists consist of commercially available components, simply mixed with one of two novel, readily synthesized methacrylate-based monomers, and lead to mechanically stable, protein-repellent micrometer-sized structures. Importantly, the distinct patches of these structures can be simultaneously functionalized with various functional molecules, such as halogenated markers and fluorescent dyes, as well as proteins in an orthogonal process under UV light irradiation. Our newly established, facile, and powerful method bears great promise for various applications, such as targeted cell attachment onto 3D microscaffolds.

Acknowledgements

C.B.-K., M.B., and G.D. acknowledge funding from the German Research Council (DFG). We thank Dr. Michael Bruns for additional ToF–SIMS measurements and Johannes Kaschke for SEM images. G.D. thanks the German Federal Ministry of Research and Education (BMBF) for funding. C.B.-K. and M.B. additionally acknowledge continued support from the BIFTM program of the Helmholtz association.

Keywords: cycloaddition · direct laser writing · microstructures · patterning · photochemistry

How to cite: *Angew. Chem. Int. Ed.* **2016**, *55*, 3817–3822
Angew. Chem. **2016**, *128*, 3882–3887

[1] F. Xia, H. Wang, D. Xiao, M. Dubey, A. Ramasubramaniam, *Nat. Photonics* **2014**, *8*, 899–907.
[2] a) K. Maex, M. R. Baklanov, D. Shamiryan, F. Lacopi, S. H. Brongersma, Z. S. Yanovitskaya, *J. Appl. Phys.* **2003**, *93*, 8793; b) C. X. J. Liu, X. Dai, L. Jin, W. Zhou, C. M. Lieber, *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 6694–6699.
[3] a) A. M. Greiner, B. Richter, M. Bastmeyer, *Macromol. Biosci.* **2012**, *12*, 1301–1314; b) C. A. Deforest, K. S. Anseth, *Annu. Rev. Chem. Biomol. Eng.* **2012**, *3*, 421–444.
[4] O. L. M. C. Wanke, K. Mueller, Q. Wen, M. Stuke, *Science* **1997**, *275*, 1284–1286.
[5] C. Wendeln, B. J. Ravoo, *Langmuir* **2012**, *28*, 5527–5538.
[6] Y. Chen, A. Pépin, *Electrophoresis* **2001**, *22*, 187–207.
[7] Y. Xia, G. M. Whitesides, *Angew. Chem. Int. Ed.* **1998**, *37*, 550–575; *Angew. Chem.* **1998**, *110*, 568–594.
[8] J. T. Fourkas, J. S. Petersen, *Phys. Chem. Chem. Phys.* **2014**, *16*, 8731–8750.
[9] a) C. N. Lafratta, J. T. Fourkas, T. Baldacchini, R. A. Farrer, *Angew. Chem. Int. Ed.* **2007**, *46*, 6238–6258; *Angew. Chem.* **2007**, *119*, 6352–6374; b) S. Maruo, J. T. Fourkas, *Laser Photonics Rev.* **2008**, *2*, 100–111; c) M. Deubel, G. Von Freymann, M. Wegener, S. Pereira, K. Busch, C. M. Soukoulis, *Nat. Mater.* **2004**, *3*, 444–447.
[10] K. K. Seet, V. Mizeikis, S. Matsuo, S. Juodkazis, H. Misawa, *Adv. Mater.* **2005**, *17*, 541–545.
[11] S. P. Klein, A. Barsella, H. Leblond, H. Bulou, A. Fort, C. Andraud, G. Lemercier, J. C. Mulatier, K. Dorkenoo, *Appl. Phys. Lett.* **2005**, *86*, 211118.
[12] B. B. Xu, Y. L. Zhang, H. Xia, W. F. Dong, H. Ding, H. B. Sun, *Lab on a chip* **2013**, *13*, 1677–1690.

[13] a) F. Klein, T. Striebel, J. Fischer, Z. Jiang, C. M. Franz, G. Von Freymann, M. Wegener, M. Bastmeyer, *Adv. Mater.* **2010**, *22*, 868 – 871; b) F. Klein, B. Richter, T. Striebel, C. M. Franz, G. Von Freymann, M. Wegener, M. Bastmeyer, *Adv. Mater.* **2011**, *23*, 1341 – 1345.

[14] L. Li, R. R. Gattass, E. Gershgoren, H. Hwang, J. T. Fourkas, *Science* **2009**, *324*, 910 – 913.

[15] J. R. Tumbleston, D. Shirvanyants, N. Ermoshkin, R. Januszie-wicz, A. R. Johnson, D. Kelly, K. Chen, R. Pinschmidt, J. P. Rolland, A. Ermoshkin, E. T. Samulski, J. M. Desimone, *Science* **2015**, *347*, 1349.

[16] a) T. Pauloehrl, G. Delaittre, V. Winkler, A. Welle, M. Bruns, H. G. Börner, A. M. Greiner, M. Bastmeyer, C. Barner-Kowollik, *Angew. Chem. Int. Ed.* **2012**, *51*, 1071 – 1074; *Angew. Chem.* **2012**, *124*, 1096 – 1099; b) T. Pauloehrl, G. Delaittre, M. Bruns, M. Meißler, H. G. Börner, M. Bastmeyer, C. Barner-Kowollik, *Angew. Chem. Int. Ed.* **2012**, *51*, 9181 – 9184; *Angew. Chem.* **2012**, *124*, 9316 – 9319; c) T. Pauloehrl, A. Welle, M. Bruns, K. Linkert, H. G. Börner, M. Bastmeyer, G. Delaittre, C. Barner-Kowollik, *Angew. Chem. Int. Ed.* **2013**, *52*, 9714 – 9718; *Angew. Chem.* **2013**, *125*, 9896 – 9900; d) T. Pauloehrl, A. Welle, K. K. Oehlenschlaeger, C. Barner-Kowollik, *Chem. Sci.* **2013**, *4*, 3503 – 3507; e) C. M. Preuss, T. Tischer, C. Rodriguez-Emmenegger, M. M. Zieger, M. Bruns, A. S. Goldmann, C. Barner-Kowollik, *J. Mater. Chem. B* **2014**, *2*, 36 – 40; f) G. Delaittre, A. S. Goldmann, J. O. Mueller, C. Barner-Kowollik, *Angew. Chem. Int. Ed.* **2015**, *54*, 11388 – 11403; *Angew. Chem.* **2015**, *127*, 11548 – 11564.

[17] B. Richter, T. Pauloehrl, J. Kaschke, D. Fichtner, J. Fischer, A. M. Greiner, D. Wedlich, M. Wegener, G. Delaittre, C. Barner-Kowollik, M. Bastmeyer, *Adv. Mater.* **2013**, *25*, 6117 – 6122.

[18] a) A. S. Quick, J. Fischer, B. Richter, T. Pauloehrl, V. Trouillet, M. Wegener, C. Barner-Kowollik, *Macromol. Rapid Commun.* **2013**, *34*, 335 – 340; b) A. S. Quick, H. Rothfuss, A. Welle, B. Richter, J. Fischer, M. Wegener, C. Barner-Kowollik, *Adv. Funct. Mater.* **2014**, *24*, 3571 – 3580.

[19] a) C. Wendeln, I. Singh, S. Rinnen, C. Schulz, H. F. Arlinghaus, G. A. Burley, B. J. Ravoo, *Chem. Sci.* **2012**, *3*, 2479; b) A. González-Campo, S.-H. Hsu, L. Puig, J. Huskens, D. N. Reinhoudt, A. H. Velders, *J. Am. Chem. Soc.* **2010**, *132*, 11434 – 11436.

[20] E. Vedejs, T. H. Eberlein, R. G. Wilde, *J. Org. Chem.* **1988**, *53*, 2220 – 2226.

[21] P. G. Sammes, *Tetrahedron* **1976**, *32*, 405 – 422.

[22] O. Altintas, M. Glassner, C. Rodriguez-Emmenegger, A. Welle, V. Trouillet, C. Barner-Kowollik, *Angew. Chem. Int. Ed.* **2015**, *54*, 5777 – 5783; *Angew. Chem.* **2015**, *127*, 5869 – 5875.

[23] G. Delaittre, N. K. Guimard, C. Barner-Kowollik, *Acc. Chem. Res.* **2015**, *48*, 1296 – 1307.

[24] Here, we do not refer to strict orthogonality as dienophiles generated from phenacyl sulfides and dienes obtained by photoenol irradiation could clearly react in a common cycloaddition. In fact, their incorporation into the microstructures artificially leads to orthogonality by preventing their encounter.

Received: October 24, 2015
Revised: December 16, 2015
Published online: February 17, 2016

