Additive manufacturing at the micro- and nanoscale has seen a recent upsurge to suit an increasing demand for more elaborate structures. However, the integration of multiple distinct materials at small scales remains challenging. To this end, capillarity-assisted particle assembly (CAPA) and two-photon polymerization direct laser writing (2PP-DLW) are combined to realize a new class of multimaterial microstructures. 2PP-DLW and CAPA both are used to fabricate 3D templates to guide the CAPA of soft- and hard colloids, and to link well-defined arrangements of functional microparticle arrays produced by CAPA, a process that is termed “printing on particles.” The printing process uses automated particle recognition algorithms to connect colloids into 1D, 2D, and 3D tailored structures, via rigid, soft, or responsive polymer links. Once printed and developed, the structures can be easily re-dispersed in water. Particle clusters and lattices of varying symmetry and composition are reported, together with thermoresponsive microactuators, and magnetically driven “micromachines”, which can efficiently move, capture, and release DNA-coated particles in solution. The flexibility of this method allows the combination of a wide range of functional materials into complex structures, which will boost the realization of new systems and devices for numerous fields, including microrobotics, micromanipulation, and metamaterials.

1. Introduction

Additive manufacturing, or 3D printing, has become a widespread process to realize complex parts and intricate structures using a broad range of materials, from elastomers,[8] metals,[2] and glass[3] to the direct printing of emulsions,[4] cells,[5] and bacterial suspensions.[6]

When it comes to research applications, 3D printing has opened up many interesting avenues to provide new platforms for the realization of materials with high structural control. Furthermore, the ever-increasing demand for miniaturization and the desire to structure materials at the (sub)micron scale has driven the development of micro and nano-printing techniques. Among those, two-photon polymerization (2PP) 3D printing is a direct laser writing (DLW) technique that affords exquisite spatial resolution in the 100 nm range.[7] However, this miniaturization comes at the cost of a reduced choice of printable materials, typically limited to a handful of organic inks and photoresists.[8–11] In spite of the great progress, significant challenges remain. In particular, the integration and precise placement of multiple and distinct materials, for example organic and inorganic ones, in a single microscale printing process are currently elusive; some examples include the dispersion of nanoparticles within a photoresist or the post-modification of the printed structures by deposition and/or plating processes.[8,12,13] However, these approaches do not enable micrometric spatial control on the localization of the different materials, for which there is only a limited range of possible choices. Nonetheless, the combination of inorganic and organic, hard and soft components, dynamic and static materials, would enable many new directions of research, for example into metamaterials.[14] Moreover, the miniaturization of devices, for example, for microrobotics, may require that different parts of the printed structures perform different functions, for example actuation, sensing, or binding, as it is possible at larger scales, or that simply multiple functions can be combined in a single device.

The realization of complex 2D and 3D materials with precise microstructural control has conversely been at the core of large efforts in the field of particle synthesis and assembly. Colloidal synthesis routes offer a huge palette of particles of different materials, with exquisite control on shape and functionality. However, their self-assembly within large scale structures presents problems due to the necessity of controlling interactions in a very subtle and precise way, and success has been achieved only in few cases.[15,16] Moreover, the
microstructures obtained by self-assembly are mostly limited to close-packed ones following energy minimization.\cite{17-19} Therefore, composite colloidal structures fabricated via spontaneous assembly have a limited range of configurations, unless directionality is built into prescribed surface patchiness.\cite{20} However, the synthesis and robust assembly of patchy particles with different interaction symmetries is highly challenging and typically only structures comprising a few particles are obtained. To bypass these issues, the directed assembly of colloidal particles within (micro-)templates can be used.\cite{21-23} Herein, we propose an alternative strategy, which combines the best features of direct printing and of the assembly of pre-synthesized particles to produce multimaterial structures with precise composition and versatile geometry.

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Finally, we use 2PP-DLW again to print onto the deposited particles to modify their shape, or to link them into complex colloidal structures, from various “colloidal molecules” \cite{24} to larger lattices and functional “micromachines.” We term this process “printing-on-particles” and show that it can be performed on multiple types of particles and using different printing materials, from commercial resins to thermoresponsive hydrogels. Finally, we present an easy harvesting procedure for the printed structures, which allows their redispersion and use and report on the realization of temperature-responsive microactuators and magnetically driven microdevices for selective binding and release of DNA-coated particles.

2. Results

2.1. 2PP-DLW-Printed Templates for Multimaterial Colloids

We begin by illustrating how 2PP-DLW can be used to expand the range of possible colloidal structures obtained by CAPA (schematically shown in Figure 1A). CAPA is an assembly method where particles suspended in an evaporating droplet are selectively deposited inside microfabricated traps during the receding motion of the droplet’s meniscus over an array of such traps that is the template. In particular, the motion of the droplet’s meniscus is typically controlled by dragging the substrate under a pinned droplet. Evaporation causes an accumulation of particles at the meniscus and capillary forces push the particles inside the traps as the droplet moves over them, leaving them there upon depinning of the contact line \cite{21}. Capillary forces typically overcome adhesion with the substrate, and, outside the traps, the receding meniscus “sweeps away” deposited particles, leading to the selective filling of the template \cite{25}. Conventional CAPA has been widely used as a patterning tool \cite{22,23} but it was recently expanded into fabricating programmable multimaterial colloidal structures via sequential deposition steps, or sCAPA \cite{26}. In sCAPA, a precise control of the trap depth profile is required to ensure that during each deposition step only one particle is deposited. The key to sCAPA is to tune the magnitude and direction of the capillary force relative to the particle position in the trap. In particular, the optimal trap depth is found to be around the radius of the deposited colloid \cite{23,26}. This poses a challenge if one wants to assemble colloids of different sizes or different stiffness, where soft particles can be deformed during deposition. Precise placement of different particles in different parts of the trap then requires a 3D height profile of each trap, with different heights for the different colloids. However, most common photolithography techniques used to fabricate the template generate 2.5D features of a uniform depth and the presence of multiple heights in one trap would require multiple alignment, exposure, and development steps. In contrast, 2PP-DLW is perfectly suited to print structures with non-uniform height profiles and holds the key to enable our multimaterial colloidal structures.

In particular, here we demonstrate the realization of hybrid colloidal molecules comprising soft responsive hydrogel particles (microgels) and hard polystyrene particles that could not be attained with 2.5D trap designs, which can be used only to assemble dumbbells and other simple shapes \cite{27}. The range of structures that can be obtained with 2PP-DLW-printed traps is significantly expanded, as shown in Figure 2.

Here, we use polystyrene (PS) particles with a 2 μm diameter and poly(isopropyl-acrylamide) (PNIPAM)-based microgels with a red-fluorescent poly(trifluoromethyl methacrylate) (PTFMA) core (see Experimental Section for details). The PNIPAM-based microgels also have a nominal hydrodynamic diameter of 2 μm, however they are deformed during capillary assembly and can squeeze into traps of much smaller size.

For the successful co-assembly of the two particle types, we have designed masters with a 1.5 μm tall cylinder with 2.4 μm diameter that fits the larger polystyrene colloids. Around this cylinder 1.2 μm wide, 1.5 μm long, 0.8 μm tall, rectangular cuboid “arms” are placed that can fit the microgels (Figure 2A; CAD drawings can be found in Section S1.1.

**Figure 2.** 2PP-DLW-printed traps for sCAPA of soft-hard colloidal clusters. A) SEM image of traps printed with 2PP-DLW for with 180° three-particle clusters. B) Bright-field + fluorescence microscopy images of clusters 2 μm PS particles and two 2 μm PNIPAM microgels with red fluorescent cores with different angles between the microgels. C) Bright-field + fluorescence microscopy images of clusters with multiple 2 μm PNIPAM microgels with red fluorescent cores and 2 μm PS particles. Scale bars: 5 μm.
Supporting Information). These masters are then replicated into poly(dimethylsiloxane) (PDMS) to obtain the traps used in sCAPA. By changing the number of arms and their relative positions, a host of different complex colloidal molecules are conceived and realized (Figure 2B,C). These hybrid structures can be directly used to obtain responsive microswimmers[27] or colloidal molecules.[28] Trap designs with arms of different sizes allow for the co-assembly of multiple different microgels, that is, for microswimmers with multiple dynamical states.[29]

2.2. Printing-On-Particles: 1D, 2D, and 3D Structures

The next step is to directly use 2PP-DLW in combination with sCAPA to print multimaterial structures. In contrast to printing whole colloidal-scale objects as monolithic pieces composed only of the printed resin,[32,33] sCAPA enables the precise positioning of colloidal particles out of a vast palette of choices in prescribed arrangements and their subsequent joining by 3D-printing polymeric links to realize composite structures.

The process to link colloids is briefly described here (Figure 1B), and a more detailed description can be found in the Experimental Section and in Section S3, Supporting Information. We start with colloids presenting acrylate or methacrylate surface groups. The presence of these functional groups enables the chemical binding of the resin to the surface of the particles during the printing process, differently to the case of physical connections, for example where the particle is simply encased in a printed polymeric shell.[31,32] Strong binding between the particles and the printed links is important to ensure minimal damage during the development and harvesting steps described below. We then deposit the colloids of interest into the PDMS templates by means of CAPA or sCAPA, as schematically shown in Figure 1B (trap sizes are shown in Section S1.2, Supporting Information). This deposition method allows for the rapid patterning of arrays of colloids over cm² areas, corresponding to ~10⁶ particles, with deposition yields >99%. The array of colloids is then transferred from the PDMS template to a glass slide for 2PP-DLW by means of an adhesive water-soluble sacrificial layer (glucose–dextran 1:1). Subsequently, the colloids are immersed in the photosensitive resin (e.g., IP-L, Nanoscribe GmbH). Crucially, the sacrificial layer does not dissolve in the resin and keeps the transferred particles fixed during the printing step. After the printing process is completed, the linked particles are developed in isopropyl alcohol (IPA) for 10 min and then dried in super-critical CO₂. The sacrificial layer is unaffected by this process.

The presence of regular particle arrays enables a fully automated printing process based on an algorithm written in Python for the detection and linking of the particles. The 2PP-DLW setup (Nanoscribe GT2) uses a conventional inverted microscope to project the near-infrared (NIR) laser light required for the crosslinking of the resin and includes a camera to monitor the printing. We use this camera to take an image of a colloidal array on the substrate. Then, we employ a particle-tracking package (TrackPy[31,32]) to locate each particle in the image and perform a nearest-neighbors search to find the immediate neighbors of each particle and label them based on their relative position. Subsequently, the user chooses which one of these neighbors are to be linked and which particles are to be removed from the look-up table. Particles can be connected in a large variety of simple individual shapes (e.g., see Figure S4-C1, Supporting Information, for rectangular units, and Figure S4-C2, Supporting Information, for triangular ones) or joined into large-scale 2D lattices, as shown in Figure 3A2–B2.

Figure 3. Colloidal structures fabricated with 2PP-DLW. A) Fabrication of rectangular colloidal lattices: Optical microscopy image of 2 µm PS particles deposited in a rectangular pattern by CAPA. Inset: A zoomed-in SEM image of the same pattern (A1). SEM images of rectangular lattices linked with IP-L after CPD (A2). B) Fabrication of hexagonal colloidal lattices. Optical microscopy image of 2 µm SiO₂ particles deposited in a hexagonal pattern. Inset: a zoomed-in SEM image of the same pattern. C) SEM images of colloidal PS dimers linked with IP-L in various shapes. From straight lines (C1), to sinusoidal links (C2, C3) of varying amplitude and frequency attached to the particles. Microscopy image C2 has a 30° angle tilt. 30° tilted SEM image of out-of-plane links connecting neighboring 2 µm PS particles (C4). The discontinuities in the print correspond to the smallest polymerizable volume (voxel) in the 2PP-DLW process. The highest point of the links is 5 µm higher in the z-direction relative to the center of PS particles. The thickness of the printed lines is smaller than 0.5 µm. D) Fabricated micro-3D-tetrapods. Optical microscopy image of the fabricated standing tetrapods imaged at the bottom-plane (top particle is out-of-focus) (D1). 45°-tilted SEM image of one tetrapod: 2 µm SiO₂ particles of two planes are linked and held together with SU-8 in 3D-structures (D2). Scale bars: 10 µm.
and additionally in Figure S8, Supporting Information. The set of links is converted to a print file that can be read by the 2PP-DLW printer, such that the linking program dynamically creates files to control stage movement, image acquisition, and printing. After an initial setup and calibration, the algorithm can be used to automatically connect particles deposited by sCAPA over large areas, up to a few mm².

After the printing, the samples are developed in IPA and dried in supercritical CO₂ to eliminate capillary forces during drying that can break the links. Figure 3A1–B2 show examples of rectangular and hexagonal lattices comprising 2 µm SiO₂ particles linked together with thin ≈500 nm-thick lines of IP-L polymer resin after critical point drying (CPD). These structures are smaller than 10 µm in size and have a characteristic “ball and stick molecule” appearance, suggesting analogies with so-called “colloidal molecules” (further examples of colloidal molecules can be found in Section S5, Supporting Information).

We then expand on this basic process by printing continuous lattices, of which we control the symmetry over large areas starting from particle arrays deposited via CAPA with a yield >99% over squared millimeters. Figure 3A shows rectangular arrays of PS particles linked into rectangular lattices and Figure 3B displays SiO₂ colloids deposited in a hexagonal pattern and linked into a honeycomb structure. During printing, only particles within a field of view of ≈200 µm × 140 µm can be linked and larger lattices are obtained by stitching many fields of view together.

Additionally, we show that different shapes of the links can be printed beyond the straight ones shown so far. In fact, our automated Python printing code enables full control over the laser path to make more complex shapes at high resolution. As example, we used sine waves with different wavelength and amplitudes to define the printing path (Figure 3C2,C3).

2.3. Functional Multimaterial Structures

The core advantage of combining sCAPA with 2PP-DLW is the precise incorporation of different materials, which can be extended beyond the two-materials examples reported above. In particular, sCAPA allows for the deposition of colloids of different materials in prescribed sequences and positions. Figure 4A–C shows the results of creating and linking mixed arrays of PS and SiO₂ colloids. The two types of particles are first deposited in traps of matching sizes in two deposition steps (the larger PS particles, false-colored in blue are deposited first), transferred as previously described and finally linked using IP-L into either multimaterial small “molecules” (Figure 4A) or into large arrays with a 2D rock salt arrangement (Figure 4C).

Complementarily, multiple materials can be incorporated by applying printing, and developing different resins in a sequence.[35,36] Here, we demonstrate that the same concept...
can be extended to linking colloids. Crucially, the particle adhesion layer must be resistant to developing steps of all resins in order to preserve the structures. In Figure 4B we demonstrate rectangular “colloidal molecules” linking SiO\textsubscript{2} colloids. We first printed horizontal links with IP-PDMS, a silicone elastomer-based resin, and then developed the sample. We subsequently re-immersed the sample in IP-L to print the vertical links, obtaining structures where hard and soft links are combined. As previously shown, these small-scale structures can be expanded into large lattices by connecting particles with continuous lines and stitching multiple fields of view together (Figure 4D).

Furthermore, the range of photoresist materials can be expanded beyond commercial links to use photopolymers with interesting functionalities. Here, we demonstrate that we can print links with thermoresponsive PNIPAM-co-acrylic acid (AA) hydrogels (Figure 4E). The formulations of these resists are based on the work of Hippler at al.\cite{37} and we have adapted them to be compatible with our printing-on-particles process (more details in Experimental Section). In the next section, we show that those printed structures retain their responsiveness after printing and harvesting, making them interesting for microactuation studies. Additionally, we demonstrate that “printing-on-particles” also works on a broader range of colloids with useful bulk and/or surface properties. In particular, we can link superparamagnetic colloids and colloids with a DNA surface coating into a cross-like structure (Figure 4F and Section S8, Supporting Information). The magnetic particles provide a means of actuating the structures, while the DNA surface coating has “sticky ends” that bind complementary DNA with a high specificity.\cite{38,39} Objects that strongly absorb the NIR laser, such as the magnetic particles, are easily destroyed if directly printed on. Therefore, the lines connecting these particles are printed edge-to-edge opposed to center-to-center, and include an additional support ring.

2.4. Harvesting and Manipulating Printed Structures

The deliberate choice of a glucose–dextran sacrificial layer, ultimately makes the release of the printed structures a very easy step. Simply adding an aqueous solution to the substrate dissolves the adhesion layer and releases the structures into aqueous environments for further uses. Figure 5A shows a large, homogeneous population of colloidal dimers with sinusoidal links fully released and undergoing Brownian motion in suspension (Movie S1, Supporting Information). The same harvesting process can also be extended to larger, millimeter-scale lattices (see Section S6, Supporting Information).

The functional PNIPAM-co-AA links retain their thermoresponsive behavior when released in an aqueous solution after printing. The hydrogel links swell to roughly double their size and are only faintly visible below the lower critical solution temperature (LCST). However, above the LCST the links collapse to around half their swollen length (Figure 5B; Movie S2, Supporting Information). Increasing the ratio of AA monomer shifts the transition to a higher temperature and with a smaller swelling–deswelling ratio (Figure 5B).

After release, the microstructures with magnetic and DNA-functionalized colloids can be manipulated by applying a magnetic field. In particular, the three magnetic colloids are on a diagonal axis of the structure, which allows the object to “walk” across a surface in a magnetic field rotating along the \(y, z\) axis (Figure 5C and Movie S3, Supporting Information). The two DNA-functionalized colloids on the other arm are coated with a DNA sequence \(A\), and selectively bind smaller free colloids functionalized with the complementary DNA sequence \(A’\) as the “micromachine walks” on the substrate (Figure 5D and Experimental Section). The DNA-bond is highly specific to the DNA sequence on the target colloid (Section S8.4, Supporting Information) and can be dissociated by simply heating the structures above the melting temperature of the DNA “sticky ends” (Figure 5E) to release the collected cargo.\cite{38,39}

3. Conclusions and Discussion

We have demonstrated that the combination of 2PP-DLW and CAPA is a powerful and versatile option to expand the palette of multimaterial microstructures obtained via additive manufacturing. In particular, 2PP-DLW plays two roles in this process: first, it enables the realization of traps with 3D profiles for the co-assembly of microparticles with different dimensions and mechanical properties during sCAPA, and then is used for the direct printing of links that connect particles in specific 1D, 2D, or 3D structures, from single units to millimeter-scale lattices. The direct printing on particles thus enables the incorporation of multiple materials, which can combine thermoresponsive, superparamagnetic, and (bio)-chemical binding properties, among others. This result enables realizing new composite colloids that can exhibit multiple internal states, as a key requirement toward obtaining reconfigurable active units with adaptive dynamics.\cite{29} The second, and more innovative role of 2PP-DLW is its use for the direct printing of links that connect particles. This approach allows overcoming major limitations in the fabrication of multimaterial microstructures either by direct printing or particle assembly alone. Concerning the former route, direct printing on pre-assembled particles unlocks the exciting possibility to incorporate precisely synthesized and functionalized units into printed microstructures, which can combine thermoresponsive, superparamagnetic, and (bio)-chemical binding properties, among others. In relation to the latter technique, printing on particles makes it possible to tremendously increase the flexibility in the design and realization of particle-based microstructures that would be impossible to realize by simple or sequential assembly.\cite{26} Summarizing, the new process flow that we report here enables us to create microstructures with unparalleled control over their geometry, composition, and surface properties with exciting potential for further innovations.

The level of control of 2PP-DLW comes nonetheless with a compromise on the scalability of the printing-on-particles process. The 2PP-DLW used in our study (Nanoscribe Photonics GT2) manufactures masters for sCAPA traps at a rate of \(10^4\) traps per hour and it therefore is significantly slower than more traditional maskless photolithography processes, which
can produce up to $10^6 \text{ h}^{-1}$. The current printing speed is still sufficient to prepare a full sCAPA master within 16 h in an overnight print, but scaling up to greater production volumes remains challenging. A possibility for up-scaling is the replication of printed masters into long-lasting replicas, for example, out of epoxy, such that many PDMS templates can be fabricated and filled in parallel. Similarly, the printing-on-particles process is automated via a custom-written Python software and it allows printing links at a rate of $10^5 \text{ h}^{-1}$ with high reproducibility. However, the identification of the tracking parameters and the printing calibration has to be optimized for each different particle/resin system. In the future, the printing speed may be accelerated exploiting recent developments in holographic techniques that split the laser beam to allow parallel printing\[40\] and a fully automated tracking and printing protocol is desirable (see Supporting Information for more details on the printing times). Therefore, we see a clear analogy with macroscale additive manufacturing, which is best suited for rapid-prototyping and small-scale production rather than for mass production and commercialization. The extension of our fabrication route to true 3D structures by stacking of particle layers could bring large freedom in the design of composition, shape, and functionality of complex structures. However, this process is still in the early phases of development, where we foresee that automated alignment and printing steps will enable a forward leap in the near future. Capillary assembly has been shown to work with particles in the sub-100 nm range,\[41\] albeit with additional complications in order to achieve the high yields reached in the microns range.\[42\] Therefore, given the ever-increasing printing resolution of 2PP-DLW, we envisage that the process can be applied at smaller scales. However, the presented workflow requires the optical detection of individual particles in order to link them, such that they must be further apart than the diffraction limit of the microscope. Consequently, we currently set the limit for reliable printing at particles on the order of 500 nm in diameter and it would be interesting to see if this limit can be challenged with alternative approaches.

Figure 5. Functional colloidal microstructures. A) Combined bright-field and fluorescence microscopy image of harvested colloidal dimers consisting of two 2 \( \mu \text{m} \) PS particles (black) connected by sine-wave IP-L links (white) after release in water. Inset: Magnified colloidal dimer. B) Thermal response of colloidal dimers connected by 5% AA and 10% AA-PNIPAM links after release in water. The graph shows the length of the link $L$, normalized by its initial length $L_0$, as a function of temperature from 22 to 35 °C. The error bars indicate the standard deviation of the measured length over at least five particles. The optical microscopy images on the left show the reversible shape changes of 5% AA-PNIPAM links below (22 °C—purple) and above (34 °C—yellow) the polymer’s LCST. C) “Walking” motion of a magnetic and DNA-functionalized “micromachine” released in water under an applied magnetic field. (Rotating magnetic field in the Z-Y plane of 50 mT at 2 Hz). D) Bright-field and merged fluorescence optical microscopy images of a “micromachine” showing the capture of multiple 1.3 \( \mu \text{m} \) target colloids with complementary DNA strands (orange). The capture is selective on the 2.8 \( \mu \text{m} \) DNA-coated silica particles, that is, “sticky hands.” E) Bright-field and merged fluorescence optical microscopy image showing the release of the captured complementary DNA-colloids after heating above the melting temperature of the DNA bonds. Scale bars: 10 \( \mu \text{m} \).
With the ability to print on particles, we can easily incorporate and localize different functions within microdevices. In the future, we envisage that our method could be used to build (soft) micro-mechanical machines, with responsive links as actuators. Currently, the particles serve as anchoring points for PNIPAM-based actuators, but they could also serve as functional parts, for actuator assembly or manipulation. If these microactuators have sequential and/or orthogonal triggers, such as light, temperature, or chemicals, one could control each link individually and create devices with programmable responses.[1,8,13,29,37,43-46] The wide variety of available colloidal particles will in the future enable us to encapsulate drugs, catalyze specific reactions, or selectively bind bio-molecules, micro-organisms, or pollutants for a broad range of applications, where the integration of the materials used for the links and the particles can complement each other in both form and function.

Finally, from a more fundamental perspective we expect a broad use of the printing-on-particles process to fabricate tailored asymmetric colloidal molecules for small-scale assembly and to realize complex functional microswimmers with a high degree of control over composition and the trajectories.[27,29,30,36,45-47] Intriguingly, the larger 2D colloidal lattices present also similarities with 2D atomic materials such as graphene or MoS₂. We thus envisage that they can provide interplaying analogies to study phononic transport and mechanical behavior at a larger scale, while providing new opportunities to design 2D colloidal metamaterials.[48]

In conclusion, we believe that combining CAPA and 2PP-DLW for printing-on-particles opens up exciting possibilities for future research and adds a new tool in the palette of available small-scale additive manufacturing processes with multiple materials.

4. Experimental Section
All chemicals, if not stated otherwise, were used as provided by the supplier. Acrylic acid (AA), N, N′-methylenebis(acrylamide) (BIS), 2,2,3-trifluoroethyl methacrylate (TFMA), sodium dodecyl sulfate (SDS), potassium persulfate (KPS), trichloro(1H,1H,2H,2H-perfluorooctyl)silane, hydrogen peroxide, 3-(trimethoxysilyl)propyl methacrylate, Triton X-45, Diphenyl(2,4,6-trimethylbenzoyl)phosphineoxide (TPO), 1,2-propanediol, fluorescein o-acrylate and Nile red were purchased from Sigma-Aldrich. N-Isopropylacrylamide (NIPAM) was purchased from Sigma-Aldrich and purified by recrystallization in Tolouene/Hexane 50:50.

Microgel Synthesis: The microgels with fluorescent cores and thermoresponsive shells were prepared in a two-step synthesis:[49] first the PTFMA-cores containing a fluorescent dye were prepared using free radical emulsion polymerization, then a shell of PNIPAM and PAA copolymer was grown by free radical precipitation polymerization around these cores. For the cores, TFMA (10 mL; 12 g; 70.3 mmol), NIPAM (940 mg; 8.31 mmol), SDS (30 mg; 0.104 mmol), and Nile red (5 mg; 0.016 mmol) were added in milli-Q water (30 mL) and stirred at 600 rpm for 20 min and purged with N₂. The reaction mixture was heated up to 70 °C. KPS (25 mg; 0.093 mmol), previously dissolved in water (2.5 mL), and stirred for 15 min at 70 °C. After 4 h of stirring at 70 °C the reaction was stopped by contact with air. The resulting particles were directly filtered. The purification of the particles was carried out via centrifugation followed by decantation, addition of Milli-Q water, and particle redisperision. The purification via centrifugation was performed three times. To remove SDS residues, the redispersed particles were dialyzed in water for 24–72 h (dialysis tube membrane: 12–14 kDa). The particles were analyzed with dynamic light scattering (ZetaSizer Nano DLS).

The PNIPAM-co-AA shells were grown by first dissolving NIPAM (1.15 g), BIS (11.5 mg), and AA (88.5 µL) in 50 mL milli-Q water. Then 300 µL of the core particle suspension was added. The mixture was stirred at 600 rpm for 20 min and purged with N₂. The reaction mixture was heated up to 70 °C and KPS (25 mg; 0.093 mmol) was slowly added. After 2 h, the reaction reached completion and the microgel suspension was filtered and dialyzed for 48 h (dialysis tube membrane: 12–14 kDa). The particles were characterized with DLS.

sCAPA Trap Design: Traps of different sizes and shapes were designed with a CAD program (Rhinoceros 3D). For CAPA depositions of 2 µm SiO₂ and 2 µm PS particles in rectangular pattern, the traps were prepared in width, 4.2 µm in length, and 1 µm in height. CAPA traps designed for the deposition of 2 µm SiO₂ as well as 2 µm PS particles in hexagonal patterns had the dimensions: 1.25 µm in length, 1 µm in height, and 2.5 µm in width. Longer sCAPA traps were designed for the double deposition of 2 µm PS and 2 µm SiO₂ in opposite directions. These traps were composed of a cuboid and semi-cylinders on both ends of the cuboid. The dimensions of the trap were: 0.7 µm in height, 10 µm in length, and width of 1.2 µm. Double-deposition sCAPA traps for 3 µm PS and 2 µm SiO₂ were designed by combining single-particle traps for 3 µm PS and 2 µm SiO₂ in an array. The single-particle traps for 3 µm PS had the same shape as the 2 µm SiO₂ particles, with dimension of: 2 µm in length, 1.5 µm in height, and 4 µm in width. The schemes for each type of trap with dimensions are found in Section S1.2, Supporting Information.

Printing sCAPA Traps with NanoScribe: A fused silica substrate (Multi-Dil, NanoScribe GmbH) was cleaned with the standard procedure from NanoScribe (rinsed with EtOH, plasma-treated for ~30 s). A commercial direct laser writing setup (Photonic Professional GT2, NanoScribe GmbH) with a 63×, NA = 1.5 with commercial Dip-in resin (IP-Dip, NanoScribe) was used for template fabrication. The print was developed with a standard procedure (20 min in PCMEA, 10 min in IPA, rinsed with IPA) and post-cured under UV light (306 nm) for at least 1 h. To decrease the adhesion of glass substrate and the PDMS, Trichloro(1H,1H,2H,2H-perfluoroctyl)silane was coated on the substrate by means of chemical vapor deposition, by exposing the substrate to a 100 µL droplet of the silane for 30 min and rinsed with isopropanol afterward. PDMS (SYLgard 184 silicone elastomer, Dow Chemicals) was poured over the silica substrate and cured overnight at 65 °C. Finally, the cured PDMS was removed from the master and used as a template for sCAPA deposition.

Particle Functionalization and sCAPA Deposition: The 2 µm silica (SiO₂) particles (Microparticles GmbH) were functionalized with methacrylate groups to enhance adhesion between colloids and the printed structure. The functionalization was performed as follows. First, 500 µL of 2 µm aqueous SiO₂ particle solution at 5 w/w% were repeatedly centrifuged and redispersed in MilliQ water. Afterward, 500 µL of hydrogen peroxide and 500 µL of ammonia solution (25% w/w) were mixed with the particle suspension for 10 min in an oil bath at 70 °C. This suspension was again washed with MilliQ water and brought to a volume of 1 mL. The solution was stirred overnight with 4 mL of dry ethanol (Sigma-Aldrich, >99.8%) and 25 µL of 3-(trimethoxysilyl)propyl methacrylate and finally washed again by centrifugation and redispersed with Milli-Q water. The 2 and 3 µm polystyrene particles were already functionalized upon purchase (Micromod Partikeltechnologie GmbH).

Before deposition, the functionalized particles were dispersed in a deposition solution composed of Triton X-45, SDS (>99.8%), Milli-Q water, (0.08 M) NaOH, and glycerol, to a concentration specified in Supporting Information. Suspensions of different particles were freshly prepared prior to each deposition and sonicated for 10 min to avoid agglomeration. Around 60 µL of deposition suspension was added over the PDMS template and dragged along at a constant speed by a flat PDMS slab at a speed of 3–5 µm s⁻¹ and at a temperature of 25 °C. sCAPA in long traps was done by depositing suspensions of 2 µm SiO₂ particles in one direction and 2 µm PS particles in the opposite direction. Deposition of mixed arrays of 3 µm PS and 2 µm SiO₂ was done by depositing the 3 µm PS particles and subsequently the 2 µm SiO₂ in the same direction.
Fabrication and Harvesting of Microstructures Printed with IPL: A borosilicate glass substrate (30 mm, Thermo Scientific) was rinsed with ethanol and plasma treated. A solution of dextran (20 w/v%) and glucose (20 w/v%) was dissolved in Milli Q water and spin-coated at 4000 rpm for 15 s. The thickness of the spin-coated layers was ~300 nm, as measured by atomic force microscopy (AFM) (Dimension icon, Bruker). After spin coating, the PDMS template with the deposited particles was pressed and gently peeled off to transfer the particles onto the glass substrate. A drop of IP-L resin was placed on the substrate and covered the region of the transferred particles. A drop of immersion oil was placed on the opposite side of the glass and the printing was done in oil-immersion mode with a 63× NA 1.4 objective. The laser power and corresponding line width are shown in Section S4, Supporting Information. After printing, the prints were washed with IPA and critical point dried (EM CPD3000, Leica Microsystems). For harvesting, a microscope spacer was placed around the critical point dried prints and closed off with a smaller glass slide (10 mm, Thermo Scientific) forming a cavity. Successively, 10 µL Milli Q water was pumped into the cavity to dissolve the glucose–dextran layer and release the printed structures. The harvesting procedure was recorded with an optical microscope (Eclipse Ti-2, Nikon).

Printing 3D Structures with SU-8: 2 µm functionalized silica particles were transferred from a filled PDMS CAPA template onto a borosilicate glass substrate with the same method mentioned above. Then SU-8 TF 6005 (Kayakli Advanced Materials Inc.) was spin-coated with at 2000 rpm for 30 s (standard procedure from microchem.) and substrate was soft-baked for 5 min at 110 °C on a hotplate. Then a second PDMS CAPA template, also containing 2 µm silica particles, was placed on top of the SU-8 layer, above the region where the first particles had been transferred, and pressed onto the SU-8. The second PDMS template was then gently peeled off, enabling the stacking of two particle layers separated by SU-8. Knowing the thickness of the SU-8 layer, links could be printed to connect particles across in 3D. After printing, the structures were developed with the standard procedure for SU-8, with a post-exposure bake for 2 min on a 110 °C hotplate, development for 3 min in PGMEA, and a 30 s wash with IPA before critical point drying (EM CPD3000, Leica Microsystems) in CO₂.

Fabrication and Characterization of Structures with pNIPAM-Based Photoresists: 2 µm functionalized silica particles were transferred from a filled PDMS CAPA template onto a borosilicate glass substrate with the same method mentioned above. A microscope spacer was placed around the transferred particles and 6 µL of pNIPAM-based photoresist was added into the spacer covering the region with the transferred particles. The cavity was sealed with a smaller glass slide (10 mm, Thermo Scientific). The 5%AA-pNIPAM resin consists of: NIPAM (189 mg), BIS (15 mg), TPO (50 mg), 1,2-propanediol (225 µL), AA (6 µL), and fluorescein o-acrylate (0.5 mg). The 10%AA-pNIPAM resin consists of: NIPAM (179 mg), BIS (15 mg), TPO (50 mg), propylene glycol (225 µL), AA (14.4 µL), and fluorescein o-acrylate (0.3 mg). Links were printed with full laser power (100%) between two neighboring particles at a scan speed of 1000 µm s⁻¹. After printing, the prints were developed in PGMEA (Sigma-Aldrich, >99.5%) for 15 min, washed in IPA for 3 min, and dried with a stream of dry N₂. For harvesting, a microscope spacer was placed around the dried prints and closed off with a gold-coated glass slide (10 mm, Thermo Scientific) forming a cavity. Subsequently, 20 µL Milli Q water was pumped into the cavity to dissolve the dextran-glucose layer and release the printed structures. The harvesting procedure was recorded with an optical microscope (Eclipse Ti-2, Nikon).

DNA-Colloids Functionalization, Fabrication, and Magnetic Manipulation: The DNA-functionalized colloids were prepared in the following manner. The protocol was based on ones found in the literature [ref. 39]. In the procedure, double-stranded biotinated-DNA was bound to streptavidin-modified colloids. The magnetic colloids used were 2.7 µm Dynabeads M270 streptavidin (Invitrogen) and the silica colloids were 2.7 and 1.3 µm plain silica colloids (Microparticles GmbH). The silica colloids were first modified with streptavidin and subsequently functionalized with DNA using the protocols described in Section S8.1 and S8.2, Supporting Information.

The DNA-colloids were assembled in a pattern of alternating rows using scAPA in opposite sides of long rectangular traps. The pattern was made by depositing the B-DNA magnetic colloids first and the A silica colloids second. Further details and results of the scAPA are given in Section S8.3, Supporting Information.

The DNA-colloids were linked with the same procedure as described above, with the exception to the prints on the magnetic colloids. Focusing the laser directly onto the center of a light-absorbing magnetic colloid resulted in rapid heating and destruction of the particle. Therefore, the links were printed on the edge of the magnetic colloids and then 3 µm diameter rings were printed around the colloids to improve the bonding. The printed structures were released with suspension buffer (10 mM phosphate, 50 mM NaCl, 0.5% w/w Pluronic F127) with the same cell as described above. Then a 0.25% w/w suspension of the 1.3 µm silica particles coated with A-DNA was injected into the cell.

The actuation of the magnetic structures was achieved using a small-scale arbitrary magnetic field generator consisting of eight electromagnets arranged in a single hemisphere, which was used to apply RMF (MFG-100-i, Magnebotix, Zürich, Switzerland). The system was integrated with an inverted microscope (Nikon Eclipse Ti2). Samples were positioned between the objective lens and the hemisphere of the electromagnets. The collected A-DNA colloids were released by melting of the DNA bond with a local increase in temperature generated by the light absorption of an Au-coated glass slide.

Imaging: The particles linked by various photoresists were imaged by bright-field and fluorescence optical microscopy (Nikon, Eclipse Ti-2) at various magnifications and by SEM (SU8000, Hitachi).

Supporting Information
Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest
The authors declare no conflict of interest.

Data Availability Statement
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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additive manufacturing, assembly, colloids, hybrid materials, nanolithography

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