

pattern 225). As shown by the inset cross-section, the channel 254 goes from a larger diameter to a smaller diameter. The minimum spacing between patterns necessary to generate such stepped flat surfaces is also the area required as a transition between steps, and can be calculated with the sidewall angle and the height difference between steps. A sidewall angle of approximately 85 degrees is created from medium-low grayscale tones. Grayscale tones close to the homogenization threshold generate surfaces with lower sidewall angles that may vary depending on the pattern.

[0078] FIG. 20 shows a pattern 205 that may be used to create such a channel 254. FIG. 21 shows a detail of an 8×4 swatch 206a (10%) and a 5×1 swatch 206b (60%) used to make such a pattern master 205. As mentioned, once the method of the present invention has created a three dimensional microfluidic device, the device may be used to create libraries of liquid plugs with arbitrary concentrations of chemicals, cells, etc.

[0079] The homogenization phenomenon is further enhanced by designing a mask with an array of circles filled with different patterns to fabricate a combinatorial set of polymerized structures. Each circle in the mask may be tiled with a different 8×4 swatch (swatch formed by 8×4 pixels), that differ in either average “grayscale tone” (the ratio of transparent to opaque pixels where 0% is completely transparent and 100% completely opaque) or in pixel size. Again as shown in FIG. 1, it was discovered that there is a transition where binary patterns on the mask are transferred to the photoresist as homogeneous polymerized patterns, or discrete polymerized patterns where the pixel geometry is apparent (e.g., one post per pixel). Interestingly, this transition does not depend on pixel density but instead is found to occur for a critical value of the product of  $n \times a$ , where  $n$  is the number of transparent pixels per unit area, and  $a$  is the side length of the pixel.

[0080] Specifically, if  $n \times a > 5500 \mu\text{m}^2$  per unit of patterned area (in  $\text{mm}^2$ ), the pattern is transferred as a homogeneous smooth surface (this condition may be referred to as the “grayscale homogenization threshold”). Further, if  $n \times a < 3000 \mu\text{m}^2$ , it is transferred as a collection of discrete pixelated patterns (FIG. 2). Thus, while the relation between grayscale tone and polymerized feature height is reproducible, it may be complex to predict. Nevertheless, as shown in FIG. 34 a simple calibration method may be used to empirically determine this relation for a set of swatches and design microfluidic devices a posteriori. For example, each swatch produces a specific photopolymerized structure of a distinct height, and, therefore, they may be used as building blocks in a hierarchical design approach for the creation of complex polymerized patterns within the device. In this way, multi-level flat features can be easily fabricated by designing adjacent large areas with swatches of different grayscale tones.

[0081] FIGS. 22-24, show how another embodiment of the present invention may be formed utilizing hierarchical patterning. FIG. 22 shows a compound of concentric circles 209 of different grayscale tones in pattern 205. The 8×4 swatches 206 below from left to right correspond to a 35%, 45%, 60%, and 65% grayscale tone. FIG. 23 shows a mask design 207 pixelated using first-level 8×4 swatches 206. First, a horn 210 is constructed from concentric circles 209 patterned with different tonalities of first-level grayscale 8×4 swatches. Such a single horn 210 is shown in FIG. 24. In any event, the circles 209 vary monotonically from black in the outer circle (1 mm outer diameter) to white in the inner circle (50  $\mu\text{m}$  diameter),

as shown in FIG. 22. Next, this design is used to define a second-level swatch, and apply it to pattern a large rectangle with the same repetitive motif as shown in FIG. 25A to create a master. Additional first-level swatches may be added to the design to generate multilevel micro channels or other curved surfaces. Alternatively, the master horn pattern 256 may be used to construct microfluidic ejectors 270, shown in FIG. 27A.

[0082] Fabrication of the ejectors 270 is as follows: an adhesive 262 is poured over the master 256, next a glass slide 264 with a thick membrane of polydimethylsiloxan (PDMS) 266 is pressed against the master 256 and the adhesive 262 is exposed to a UV light 261. When both sides are pressed together, the tips of the horns are inserted into the soft PDMS layer 266 to form an ejector plate 272. Thus, the horn cavities 269 created on one side of the sandwiched membrane end up in orifices that surface on the other side of the membrane. Next the completed membrane or ejector plate 272 is released from the master. The membrane with the horn cavities 269 connecting both sides is used as an ejector plate.

[0083] A prototype of an atomizer 274 with an ejector plate 272 is shown in FIG. 26. The plate 272 is mounted over a PDMS gasket 282 and piezoelectric actuator 284. These are then assembled between pieces of aluminum and polycarbonate to form a sandwich structure 286 around a fluid cavity, as shown in FIG. 26.

[0084] To operate the ejector, the fluid cavity is primed with water. A sinusoidal AC voltage signal is then generated by a function generator provided by Stanford Research Systems DS345 and an RF amplifier provided by T&C Power Conversion AG1020. When it is operated at a specific frequency (e.g. from 0.8 to 1.1 MHz), the piezoelectric transducer 276 produces standing acoustic waves that are focused by geometrical reflections within the horns, creating a pressure gradient that can be used for fluid jet ejection. The resulting microfluidic device 274 may be used to eject liquids, such as water, through the thiolene nozzle orifices at  $\approx 5 \text{ ml/min}$  flow rate (see, e.g., FIGS. 27A and 27B). Moreover, the diameter of the nozzle orifices (40  $\mu\text{m}$ ) is well suited to cell manipulation via focused mechanical forces to enable various biophysical effects such as the uptake of small molecules and gene delivery and transfection. Additionally, the grayscale mask here may be designed to create nozzle orifices of different sizes for application to areas as diverse as mass spectrometry, fuel processing, manufacture of multilayer parts and circuits, and photoresist deposition without spinning.

[0085] FIG. 25A illustrates the result when the design of a single horn shown in FIG. 24 is used as a second-level swatch to pattern a large rectangular area (20×20  $\text{mm}^2$ ). After fabrication, this swatch pattern may be used to generate an array of thiolene horns. As shown in FIG. 25B, these horns then may be used as a template to replicate repetitive cavities and form an ejector plate (see, e.g., FIG. 26).

[0086] FIG. 26 shows a microfluidic device including the ejector formed from the array of horns. FIG. 27A shows a schematic illustrating the operation of an ultrasonic atomizer created using a method of the present invention. Here fluid enters the chamber through a capillary. When the piezoelectric transducer is driven at a resonant frequency of the chamber, pressure wave focusing leads to ejection of jets of liquid. FIGS. 27A and 27B both show a demonstration of jet ejection with a microfluidic.

[0087] As shown in FIGS. 28 and 29, various pixels of varying sizes may be used to create a wide variety of swatches