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MANUFACTURING OF MICROWELL USING OPTIMIZED PROCESS PARAMATERS IN PHOTOCHEMICAL MACHINING PROCESS

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Abstract
Photochemical machining (PCM) is an upcoming trend in the field of non-conventional machining methods, especially in micromachining for very thin components. PCM is widely used in aerospace, automobile, biomedical and electronic industries. SS316L is a biocompatible material used in the manufacturing of different medical devices. In the current study, the Grey Relational Analysis (GRA) approach is used to improve the performance parameters, such as the etchant temperature, concentration, and time of etching during PCM of SS316L as substrate. Ferric chloride is used as an etchant for PCM of SS316L. Significant process parameters are evaluated with ANOVA analysis. Design of Experiments (L_9) orthogonal array is applied for experimentation. A confirmatory test is performed to verify the improvement in the investigations. The obtained set of machining parameters is applied to create the micro projections on the SS316L substrate. PDMS (polydimethylsiloxane) is used to develop the microwell with a size limit of depth as 21µm and radius as 126 µm. The microwell developed has applications in tracking, analysis, and diagnosis of an individual cell.

Keywords:
PCM, Micro etching, Undercut, Optimization, Microwell, Cell-tracking.

I. Introduction

Bio-MEMS, Microfluidic devices and Microsystems are showing tremendous application in the era of biological studies and medical applications. There is a continuous demand to develop miniaturized, economical, and simple systems for cell analysis. The microwell has emerged as a recent application for observation and analysis of the single cell. The microwell is a physical structure that is used to track, handle and characterize the different affected and non-affected cells, tissues, embryonic development in its initial stages of their growth [1]. Low cell capturing efficiency or inability to handle the single cell from the cell colony is a prominent lacuna in the existing techniques used [2]. Current techniques are using 2-D flat morphologies for the analysis of cells but constructions of 3-D structures are gaining significant interest due to their usefulness in understanding the reflective behavior of cells during vitro analysis (outside the body)[3]. Stem cell (ability to develop any type of cell) compromises relevant properties such as cell behavior, analysis capacity and differentiation in individual cell property in 2-D culture [4]. 3D tumour models are needs to get analyzed for better prediction in cancer and drug performance research [5]. Many bio-researchers have contributed to microwell manufacturing and cell analysis. Eun Hae Oh et al. have developed a stamp for manufacturing the microwell on the PDMS structure [2]. The array of a microwell with an individual cell with a size of 500 µm (diameter) and 100 µm (depth) is manufactured but above proposed method is costly, need a separate stamp manufacturing method and also it is difficult to control the depth of microwell. Xuefeng Qiu et al. [4] have derived a 3-D assay system based on polyethylene glycol dimethacrylate (PEGDM) microwell which is proven to be better in producing mesenchymal stem cells. Yoon Young Choi et al. [6] have attempted to manufacture the concave cell array on PDMS structure. Vacuum pressure applied through the substrate's perforations creates the micro projection on the PDMS structure. The micro projections are coated with SU-8 prepolymer solution. Inability to control the SU-8 photoresist thickness, uniform vacuum and complexity in the process limits the application of the process. Tianqing Liu et al. [5] have fabricated the microwell with ice lithographic technique. Water droplets are frozen and used for casting with PDMS. The current method suffered from limitations as need of special environment, changing the size of ice droplet and non-uniform shrinkage of PDMS. Julia Dahlmann et al. [7] have developed the standard patterns of hydrophobic silicon masters template which is used to produce patterned agarose-DMEM microwell. S.Kabiri Ameri et al. [8] have applied graphene on the glass substrate which is coated with SU-8. The microwell with dimensions (20 µm x 20 µm x 7 µm) is formed on the surface of the graphene. Sung-Hwan Moon et al. [9] have developed a PDMS microwell sheet (10 mm x10 mm x 3 mm) consisting of 100 arrayed cylinder microwell with soft lithographic technique. Xiaoshan Yue et al. [10] have fabricated micro well through the micro-molding method. Chenyu Wang et al. [11] have developed the microwell having a high capturing rate with reagent space. All above current methods have limitations with cost, environmental issues, accuracy, dimensional stability, development of PDMS stamp, complexity. PCM is recommended as a new method for manufacturing microwell as it eliminates constraints and emerges as a better process for biomedical analysis. PCM is frequently used to produce extremely thin components with complex profiles and emerged as a better substitute to conventional machining methods in different eras [12]. Photoresist and etchant are used to do the selective etching over the substrate [13]. Improvements in surface quality and MRR during PCM of St304 using TEA (triethanolamine) as an etchant have been reported by Fadaei et al. [14] Industrial etchants have performance requirements and metrics established by Allen D and Almond H [15]. Aluminum was carefully etched with FeCl3 etchant by Cakir and parametric effects are explored. SS316L is chemically etched using an aqueous ferric chloride solution [16]. PCM process has advantages as it is free from burr and stress hence no post-treatment of a specimen is needed after machining. It is a unique method for machining thin and complex profiles with minimum cost. The process can be controlled by varying machining parameter levels. The metallurgical properties of the part are unaffected after the machining. In the current findings, the primary governing response parameters of the PCM process are optimized utilizing the Multiobjective Grey Relational Analysis (GRA) method, including PCM etchant concentration, temperature, and etching time. SS316L is used as the substrate, and array of microwell is produced utilizing an optimal set of machining parameters. By performing micro etching on the SS316L substrate, the array of micro projections on SS316L is created. The PDMS and binder (10:1) are poured on micro projections to get an array of the microwell. In the second method, a lithographic process is used to photopolymerize the photoresist on an SS316L substrate. A photoresist developer is used to remove the unexposed photoresist. The exposed remaining photoresist acts as micro projections on which PDMS is cast to develop the array of the microwell. Microwell dimensions are assessed using a video measuring device (VMM) and a counter tracer (CX2). The developed microwells are recommended for different biological applications and its significance is discussed.

II. Material and Method

One of the recomamnded material is stainless steel-316L because of its low corrosion resistance, biocompatibility, improved mechanical qualities, variety of applications, and ability to be used with photochemical machining. It is used for manufacturing bio-implants and surgical blades In the current investigation, it is used as the substrate material for the etching. PDMS (polydimethylsiloxane) along with the binders is poured on the micro projections developed on SS316L substrate to create the array of micro holes. PDMS properties make it a convenient material for many micro manufacturing applications. The development of the array of microwells on the PDMS element allows for a thorough understanding of each cell's unique behavior and characteristics. The binder and PDMS are mixed and poured on the micro projections developed on the substrate. PDMS has better structural stability, non-chemical affinity, transparency, better cell handling and cultivating properties. It is one of the recommended materials for biological analysis.

2.1 Experimentation

During the PCM process, the developments of the Phototool and specimen preparation are carried out simultaneously. The experimental setup is shown in figure 1. The specimen (SS316L) is cut with the required dimension (30 mm x 30 mm). To improve the photoresist's adherence, the specimen is washed with caustic soda while being submerged in flowing water. The centrifugal photoresist coater is fitted with the specimen. The centrifugal photoresist coater's rotational speed and the amount of photoresist used determine how thick the photoresist layer is deposited.

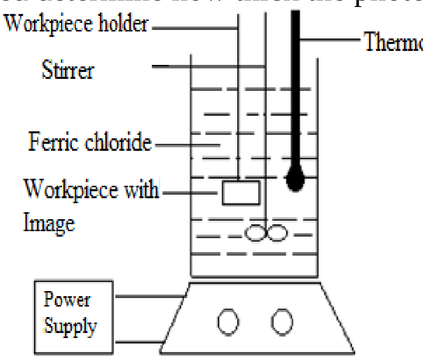

Figure 1: Experimental setup for PCM

Figure 2: Phototool used for DoE

Figure 3: Photo tool used for manufacturing of microwell

The coated specimen is dried in the dryer by hanging it inside without coming into touch with the dryer's walls. The size and shape of the artwork on the Phototool determine the maximum size that can be micromachined using the PCM. Phototool is a transparent piece of paper with the necessary artwork printed in black. The Design of Experiments (DoE) is carried out using a phototool with a black, 10 mm-diameter circle as the artwork. The artwork is created with CorelDraw (CAD) software and produced with a high DPI printer on transparent paper or lithfilm (shown in Figures 2 and Figure 3). The darkroom is where the photoresist coating and drying are done. The dried specimen is put within a sandwich-like phototool assembly. UV source is employed. By providing compressed air from the etching tank's bottom, etchant concentration is kept constant and uniform. Using the polymer net, the specimen is placed in the etching tank. Through preparatory experimentation, the set of levels for the machining parameters for the experiment design is produced. Changes are made to the machining settings to produce three duplicate sets for DoE. All safety precautions are used while doing the controlled etching. Smooth cleaning is used to remove the etchant and the exposed photoresist, and appropriate measuring tools are used to gauge the response parameters.

2.2 Grey Relational Analysis Method

Using the multiobjective GRA technique, the best set of performance parameters is obtained. In order to determine the set of ideal machining parameters, the controllable variables (etchant temperature and concentration, etching time) and major responses (MRR, Uc, and EF) are examined. The experimental analysis makes use of the DoE (L9) orthogonal array. Following experimentation, the answers are assessed using the appropriate measuring tools for each machined sample. The estimated MRR (mm^3/min) is the volume of material removed in (mm^3) per minute of machining. The difference between the thickness of the specimen before and after machining over the machined area is used to calculate depth of cut (DoC). A phototool's undercut is defined as the difference between its size (10mm) and the additional machining area (a circle with a larger diameter).

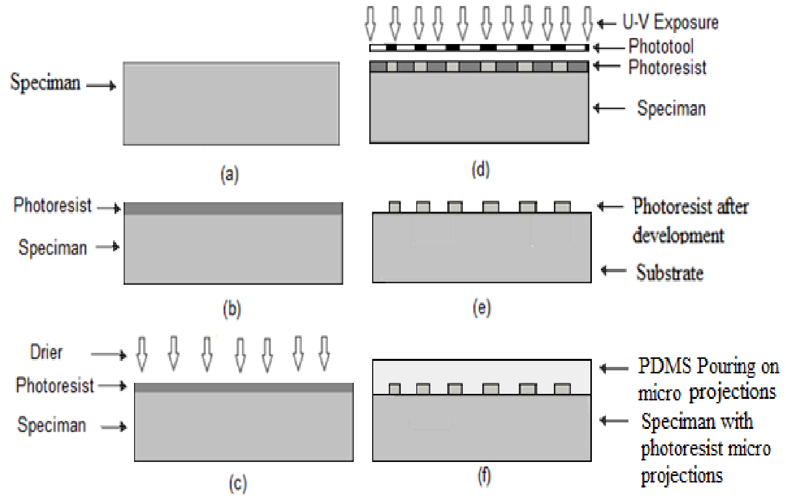

Figure 4. The fabrication of micro projections by micro etching on substrate SS316L. a: Cleaned and dried specimen b: Centrifugal photoresist coating c: Dring d:UV exposure with phototool e:Photoresist retained after development f: PDMS pouring on photoresist micro projection to manufacture the microwell.

The video tool is thought of as a real-time detecting tool. The VMM specifications are as follows (Make: Accurate gauging Pvt. Ltd, Range: 0 to 300 mm, Model: Micro vision, Resolution: 0.1 µm, L.C: 0.001mm) The undercut developed as a result of the etchant's longer contact time with the area underneath the photoresist. The etch factor (EF) determines the quality of the photochemical machining process over the substrate. EF is the proportion of Depth of Cut (DoC) to Undercut (Uc). Deviation sequence for machining parameter as (larger or smaller is better) obtained from Eq.1 and Eq.2.

$$x_i(k) = \frac{x_i^{(0)}(k) - \min x_i^{(0)}(k)}{\max x_i^{(0)}(k) - \min x_i^{(0)}(k)} \quad (1)$$

$$x_i(k) = \frac{\max x_i^{(0)}(k) - x_i^{(0)}(k)}{\max x_i^{(0)}(k) - \min x_i^{(0)}(k)} \quad (2)$$

Deviation sequence of normalized value $x_i(k)$ from the $x_0^*(k)$=1 is obtained by using Eq.3 [17].

$$\Delta 0_i\ (k) = \left|\ x_0^*(k) - x_i(k)\ \right| \quad (3)$$

For each trial, the maximum and minimum values of the deviation sequence are used to calculate the values of the Grey Relational Coefficient (GRC). ξ has the distinction value of 0.5 [18]. Eq.4 estimates GRC values.

$$\gamma(x_0(k), x_i(k)) = \frac{\Delta \min + \xi \Delta \max}{\Delta 0_i\ (k) + \xi \Delta \max} \quad (4)$$

The Grey Relational Grade (GRG), which is based on the GRA theory, is derived for determining each experiment's rank. Equations 1 through 4 illustrate the crucial phases of GRA. By employing the quality features of larger is better (MRR and EF) and smaller is better (Uc), linear normalization of the original sequence values is performed [19]. GRG is estimated by averaging the GRC values of all 9 experimental sets. The results obtained after GRA analysis shows that experiment No.9 has the highest value of Grey Relational Grade (Highest rank) from all the set of experiments. It is selected as the optimum set of machining parameters by using Grey Relational Analysis [20]. An evaluated optimum set of machining is selected for manufacturing microwell having different applications in the biological field. The results after experimentation and GRA analysis are shown in Table 1.

2.2.1 Confirmatory test

Following experimentation, the confirmatory test examines performance using an ideal set of machining parameters. Using Eq. 5, the Grey Relational Grade (GRG) is determined.

$$\Gamma_i = \Gamma_m + \sum_{i=1}^{q} (\Gamma_i - \Gamma_m) \qquad (5)$$

Table 1. Estimation of GRG and rank of experiment

Sr.No	MRR (mm^3/min)	Undercut (mm)	Depth of cut (mm)	Etch Factor	GRC (MRR)	GRC (Uc)	GRC (EF)	GRG	Rank
1	0.00080	0.1508	0.004	0.026	0.4814	0.3373	0.3366	0.3851	6
2	0.00150	0.0664	0.015	0.225	1.0000	0.5736	0.3941	0.6559	4
3	0.00140	0.0254	0.021	0.826	0.8666	0.3552	0.8119	0.6779	2
4	0.00040	0.1533	0.002	0.013	0.3714	0.3333	0.3333	0.3460	9
5	0.00120	0.0283	0.012	0.424	0.6842	0.8386	0.4746	0.6658	3
6	0.00113	0.0190	0.017	0.894	0.6372	0.3564	0.9226	0.6387	5
7	0.00020	0.0399	0.001	0.250	0.3333	0.3526	0.4024	0.3627	8
8	0.00050	0.0298	0.005	0.167	0.3939	0.3544	0.3754	0.3745	7
9	*0.00093*	*0.0150*	*0.014*	*0.933*	*0.5327*	*1.0000*	*1.0000*	0.8442	*1*

The results obtained after the conformance test are shown in Table 2. Material Removal Rate (MRR) shows a rise from 0.00073 mm^3/min to 0.00093 mm^3/min. Undercut (Uc) is decreased from 0.0210 mm to 0.0150 mm and Etch Factor (EF) shows enhancement as 0.787 to 0.933. The procedure under investigation has a 0.3851 improvement in GRG overall. Applying the set of performance criteria discovered through DoE experiments results in process improvement.

Table 2. Confirmatory test for results

	Initial Machining Parameters	Optimal Machining Parameters	
		Prediction	**Experiment**
	$A_1B_1C_1$	**$A_3B_3C_3$**	**$A_3B_3C_3$**
Material Removal Rate (mm^3/min)	0.0080	0.00073	0.00093
Undercut (mm)	0.1508	0.0210	0.0150
Etch Factor	0.026	0.787	0.933
GRG	0.3851	0.7108	0.8442
Improvement in GRG : 0.1334			

2.3 ANOVA Analysis

Analysis of Variance (ANOVA) is performed to estimate the significant process parameters (F-value) affecting the responses measured after the experimentation. From Table 3, Table 4 and Table 5. Both the etching time (in minutes) and etchant temperature are important process variables for MRR. Another important process parameter for Undercut and Etch Factor is time of etching.

2.4 Development of micro projections on a substrate for Microwell manufacturing

The SS316L micro projections are created using the two techniques listed below.

2.4.1 By etching the SS316L and developing the micro projections

In this method, a phototool (a transparent paper with the required shape) is developed as shown in figure 2 and figure 3. The array of black color circles (25 µm) is developed on the phototool with the help of CorelDraw software and a printer with high DPI. PCM stages like preparation of the specimen, development of the photoresist and UV exposures are performed in the darkroom and laboratory. A positive type of photoresist is used. In a positive type of photoresist, the photoresist which is under the exposure of UV light gets photopolymerized by breaking the covalent bonds to convert it into several monomers. The area that is exposed to UV light, or the area that is not enclosed by a black circle printed on translucent paper, is where the etching is carried out using the best possible set of machining parameters. The micro projections are observed on the SS316L substrate on the black-colored micro circle drawing after the etching. The diameter of the circular artwork on the phototool determines the size of the micro projection, and the height is determined by the time of etching as determined by the best machining settings and prior trials. After being softly cleaned away, photoresist and etchant are employed as a template for PDMS material molding.

2.4.2 By processing of Photoresist developer

PCM steps like specimen preparation, development of photoresist, drying and UV exposure are performed without the etching stage. Utilizing the centrifugal coating method, the positive kind of photoresist is applied to the substrate, and the speed of the centrifugal coating device regulates the photoresist's thickness. As discussed, the positive type of photoresist is get polymerized under UV exposure. Once it is exposed through the phototool with a black colored micro circular array, the specimen is gently rinsed in the photoresist developer (LRP) solution without etching. Weak photoresist (exposed photoresist through the phototool) is removed from the metal substrate. The micro projections of none exposed photoresist which is protected with the mask are developed on the metal substrate. The speed of the centrifugal coater and the amount of photoresist dropped on the specimen determine the height of the micro projection (of photoresist). The specimen with projection is dried to provide the strength and hardness to a micro projection of photoresist over the specimen. These micro projections developed with a photoresist having less strength as compared to micro projections of the metal substrate itself. Micro projections are developed on SS316L specimens with the above two stated methods. PDMS and cross linker (10:1) mixture is developed in a liquid state. PDMS prepolymer is then poured on the micro projections of photoresist on the substrate very gently and uniformly. The micro projections on the substrate act as the positive mould to create the array of microwell. The PDMS is cured at room temperature for 48 hours. After the curing and solidification of PDMS, the array (5 x 5) of microwell is developed. The steps for developing micro projection on SS316L substrate by photoresist development are shown (Figure 4).

III. Result and discussions

3.1 Optimization

The optimum set of machining parameters during photochemical machining of SS316L is obtained as etchant concentration (550 gm/lit), etchant temperature (40^{o}C) and etching time (15 min.). The confirmatory test shows the improvement in the response parameter by the optimum set of experimentation. The experimental findings demonstrate that the difference between the etch rates of grain borders and grain regions has an impact on the MRR. When compared to the grain surface, the grain boundaries are weaker. In comparison to grain regions, the chemical etchant removes more material from grain borders. The kinetic energy of the etchant particles changes as the etchant's temperature rises. By overcoming the activation energy, it will promote bond breakdown and more material will be removed. The molecular energy level will rise as a solution's temperature rises, resulting in more particle collisions and a higher reaction rate. The Stokes-Einstein equation ($D=K_B.T / 6\pi\mu R_0$) shows that. D (Diffusion coefficient) is directly proportional to T (Temperature). The rate of reaction increases linearly with temperature. Consequently, MRR rises as temperature rises. The etchant particles benefit from increasing diffusion as the period goes on. By lowering the diffusion distance, the contact rate between a solid (material) and liquid (etchant) speeds up the etching process. As the contact rate between the reacting atoms increases, more covalent bonds between ferric and metallic ions are broken. It has been found that MRR values rise steadily as etching time increases.

Table 3. ANOVA analysis for MRR (mm^3/min)

Source	DF	Seq. SS	F	P
Temperature (°C)	2	0.0000007	9.36	0.097
Time (min.)	2	0.0000008	10.98	0.083
Concentration (gm/lit.)	2	0.0000001	0.43	0.689
Error	2	0.0000001		
Total	8	0.0000017		

Table 4. ANOVA analysis for Undercut (mm)

Source	DF	Seq. SS	Adj. MS	F	P
Temperature(°C)	2	0.0044588	0.0022294	3.38	0.228
Time(min.)	2	0.0148239	0.0074120	11.25	0.082
Concentration (gm/lit.)	2	0.0035975	0.0017987	2.73	0.268
Error	2	0.0013181	0.0006591		
Total	8	0.0241983			

Table 5. ANOVA analysis for Etch Factor

Source	DF	Seq SS	Adj MS	F	P
Temperature(°C)	2	0.01538	0.00769	0.50	0.664
Time (min.)	2	1.02717	0.51358	33.72	0.029
Concentration (gm/lit.)	2	0.03170	0.01585	1.04	0.490
Error	2	0.03047	0.01523		
Total	8	1.10472			

The density of the active ferric ion in the solution rises as the reactant concentrations rise. The pace of the reaction tends to rise with the number of ferric ions. As atoms collide with metallic substrate more frequently, the efficiency of the. As per Fick's law ($J=-D.\ dc/dt$), J (The mass flux) is directly proportional concentration gradient. MRR values increases with rise in etchant concentration. The optimum quantity of machining parameter is to be obtained for best etching quality.

3.2 Measurements of Microwell

A Video Measuring Machine is used to observe the size of microwell over the PDMS. The rectangular array (5 x 5) with a size of microwell on PDMS is developed. Counter Tracer (XC2) made by Mahr, Germany is used to measure the dimensions of the microwell. The microprobe (Φ 0.1µm) of tracer is traveled over the inline microwell and dimensions are measured. The probe movement over inline microwell is shown in figure 5. The dimensions of the individual microwell are shown in figure 6. The depth of microwell manufactured is found to be 21µm and radius as 126µm. The size and shape have the ability to do the intended various biological applications as observed from the size of microwell from the literature. The author has manufactured the microwell with size (depth as 21µm and radius as 126 µm) and various biological applications are discussed herewith some figure mentioned herewith. Analysis of single-cell is a very significant aspect in biology to find the root cause of all diseases. Traditionally, the group of cells is tracked and placed over the flat dishes. The cell behavior is very difficult to observe under the group of cells due to overlapping and adhesion of the cell to form the cell colony. Microwell is proved to be a better structure to study individual cell from the group of cells. The cell structure under the florescent lamp with individual and group cell is shown figure 7. Microwell size and geometry should be tuned with the type of cell and the purpose of treatment.

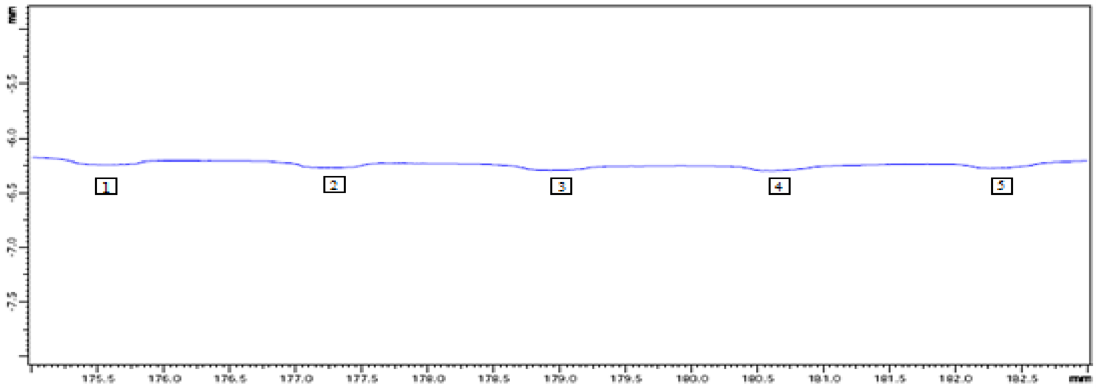

Figure 5. Path traced by Counter Tracer (XC2) made by Mahr, Germany over the five consecutive micro wells.

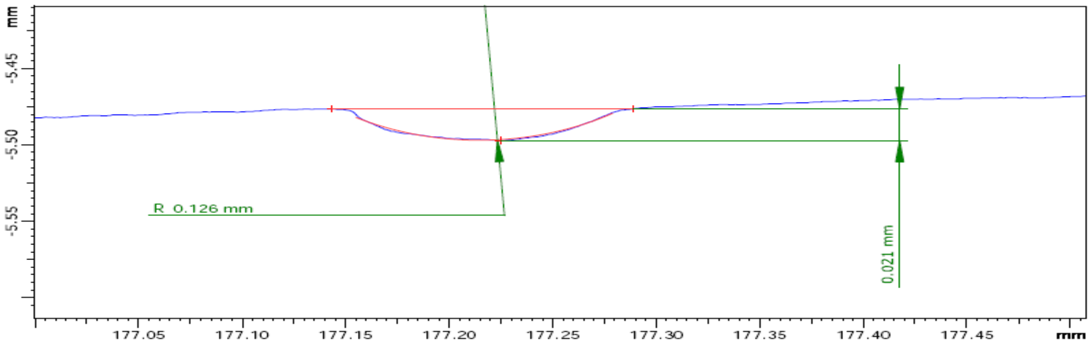

Figure 6. The dimensions of one micro well are measured by Counter Tracer (XC2) made by Mahr, Germany.

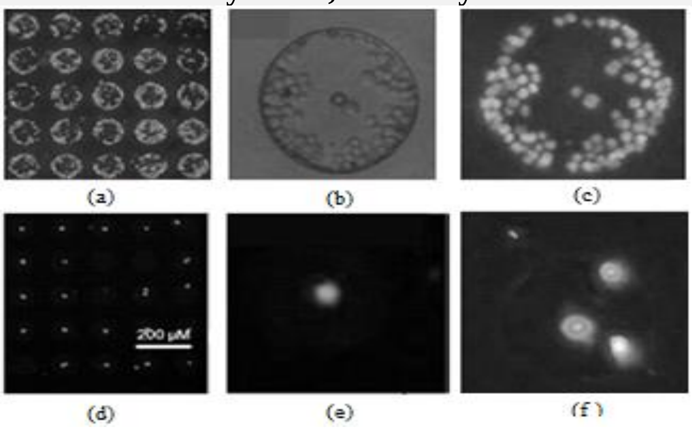

Figure 7 a: Array of micro well b: and c: Micro well with multi cell d: e: and f: multi and individual cells are observed through florescent lamp. (reprinted with permission from (Yu Y(2018)).

IV. Conclusion

According to the observations and results of the current study, the best machining parameters for SS316L include an etchant concentration of 550 gm/lit, an etching time of 15 minutes, and an etchant temperature of 40 ^{o}C when using a multiobjective optimization technique called Grey Relational Analysis. The rate of material removal keeps rising as time, temperature, and etchant concentration grow. By creating an array of microwell (5 x 5) with individual diameters of microwell as radius and depth as 126 m and 21 m correspondingly on the PDMS material using SS316L as the substrate, the capabilities of the photochemical machining process are investigated. The effective simulation of a set of microwell is developed and discussed with the capability of trapping, handling and analyzing the individual cell.

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