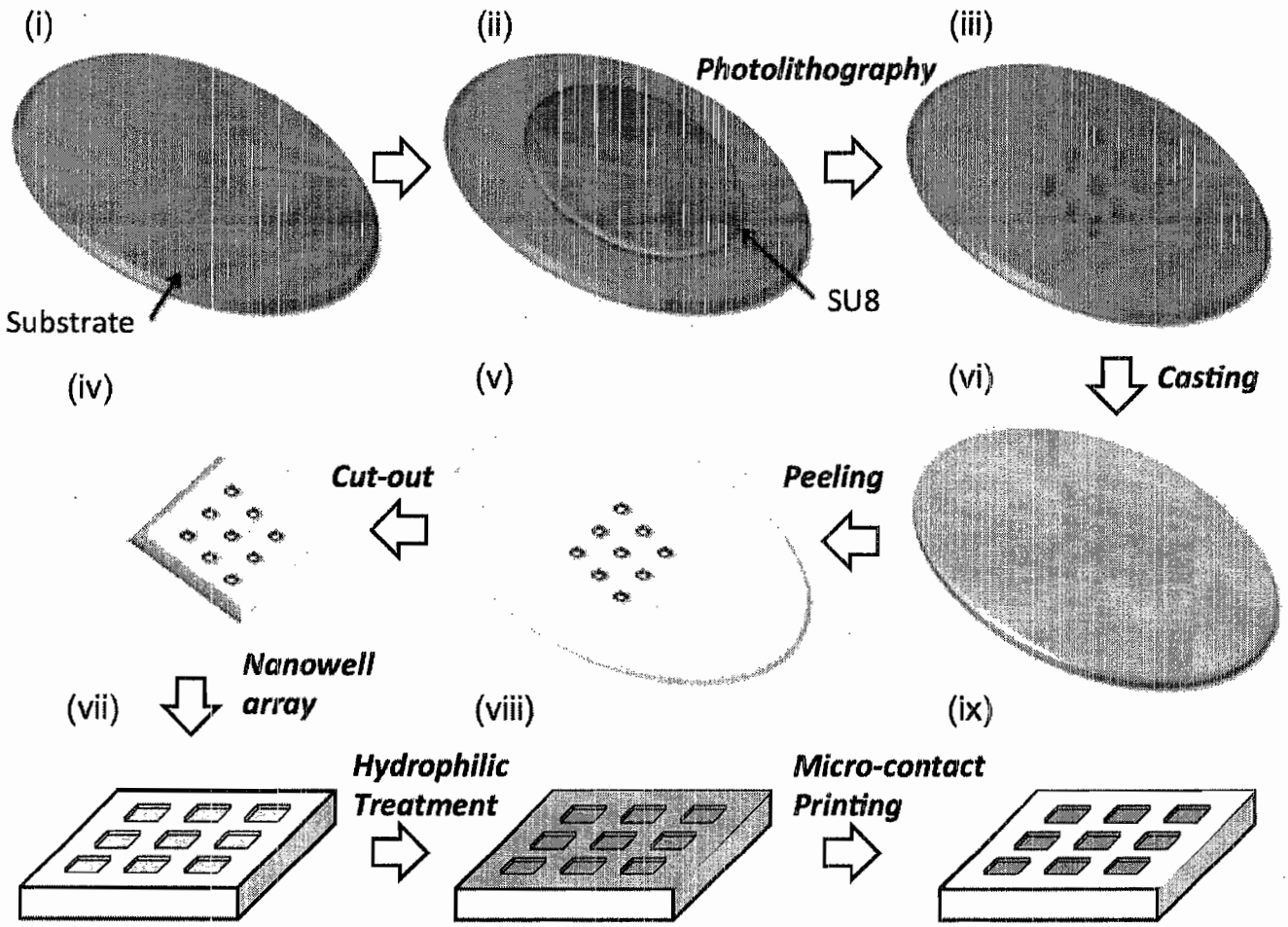
one could arrive at detection of the bacteria much faster than in a larger homogeneous sample. This is the working principle behind our fast metabolic monitoring of bacteria.

A similar segmentation can be achieved in a digital droplet microfluidic platform [29]. However it requires sophisticated instrumentation for droplet generation compared to the method here and may not be suitable for resource poor settings. Furthermore, the high permeability of the oil barrier to gases precludes it from use when the metabolic marker is oxygen.

Large scale arrays of nanowells have been used in the past for single cell imaging and analysis[30] and analyzing cytoplasmic contents of individual cells[31]. Recently, it has been used to perform end-point ELISA type immunoassays in the nanowell format for identification of mycobacteria[32]. However, ELISA requires extensive sample preparation for analysis, which is not only labor intensive but also a time-consuming. Also, endpoint assay typically requires that the assay duration be set for the minimum detectable concentration, as the concentration present in the sample is not known apriori.

Here we report a generic method that uses nanoliter well arrays to perform real-time measurement of oxygen as a metabolic marker to detect viability, growth and drug effectiveness of bacteria. The method requires very simple sample preparation that is suitable for resource poor settings. Real-time measurements allow faster detection of growth especially when the concentrations are higher than a single bacteria/well. The instrumentation required for imaging and fluorescent measurements can also be made simpler and cost-effective using some of the recent developments in low cost optics[33]. In combination, this approach provides an effective method for fast culture based detection in resource poor settings.

**(a)**



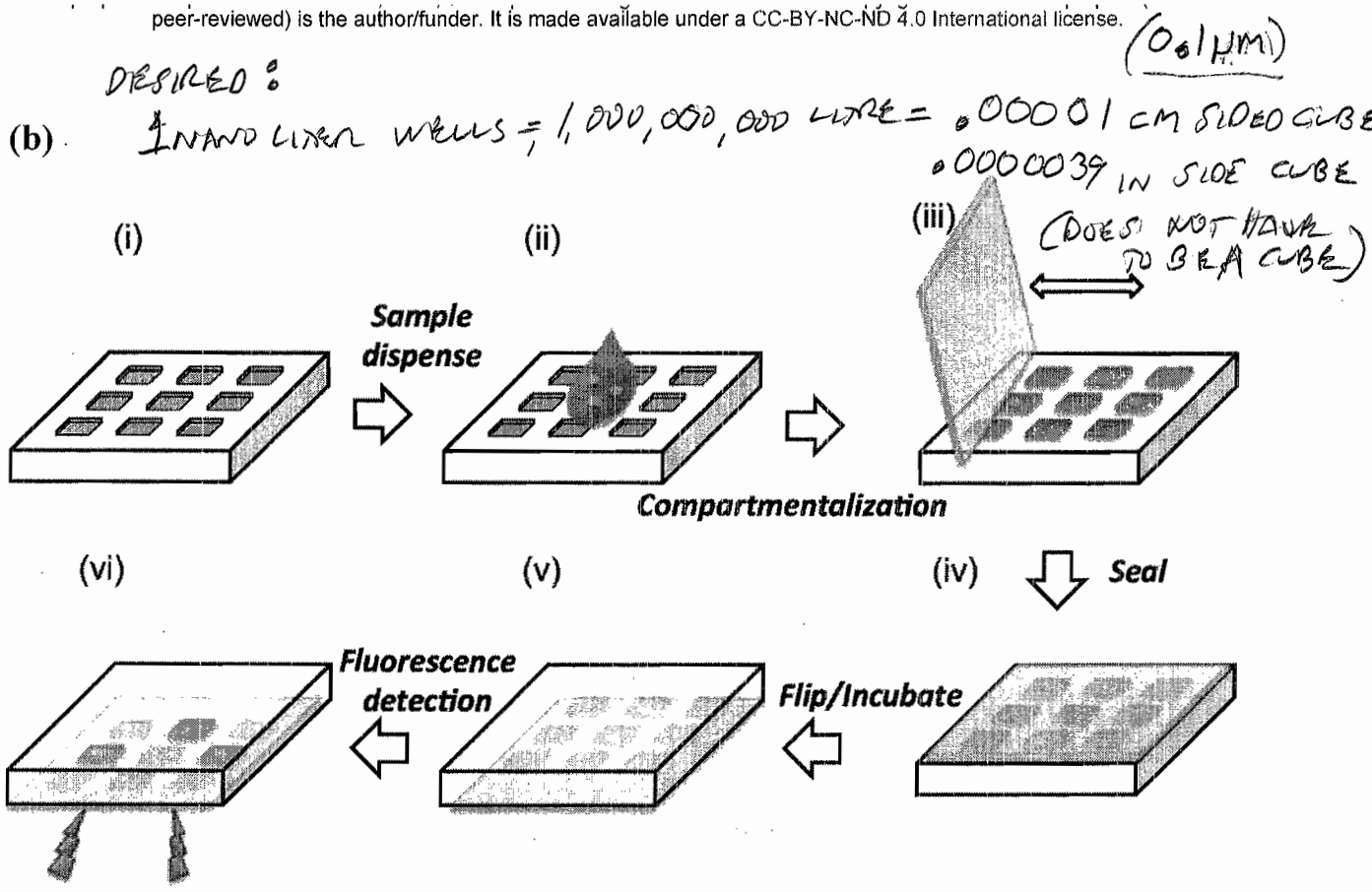



**Figure1: Schematic representation of the** (a) soft lithography and surface modification process to fabricate the device and (b) sequence of operation of the device

## 3. MATERIALS AND METHODS

**Materials**. Silicon wafers (mechanical grade, 3", 500 μm thick) were purchased from University Wafer Co., USA. Negative photoresist Su8-100 and developer were acquired from Microchem Co., USA. PDMS polymer was purchased from Dow Corning Co., Canada. The powder of RTDP (ruthenium tris (2,2'-dipyridyl) dichloride hexahydrate) was obtained from Sigma-Aldrich (#224758). Liquid microbial growth medium, Luria Broth (LB) purchased from Sigma-Aldrich (#L2542). The hydrophilic surface modification agent (N-Wet 410) was gifted by Enroute Interfaces Inc., and one part of the n-Wet 410 solution was diluted with 9 parts of toluene giving a final concentration of 10%V/V. Glass slides (Corning, 75x25x1mm) were used to sandwich our device. A commercial hydrophobic surface modification agent, Aquapel was obtained from Aquapel® Glass Treatment, and was used to make glass slides hydrophobic. Methylcellulose MC (M-0262) viscosity of 2% aqueous solution at 20 ◦C, 400 centipoises was obtained from Sigma.

**Microfabrication of nanowell array.** SU-8 100 photoresist is spun on a Silicon wafer at 1,700 rpm to produce a layer of 100-μm thickness (figure 1a-ii). This layer was patterned using photolithography and produced square or circular shapes of sizes that varied from 100-1000 $\mu m$ to produce the mold for nanowell devices (figure 1a-iii). Next, polydimethylsiloxane (PDMS) prepolymer (base: curing agent = 10:1) was cast on the mold, cured and peeled off to replicate the pattern of the mold forming an array of nanoliter wells as shown in figure 1a-iv. Next, the cast and crosslinked PDMS elastomer is peeled off and cut to the required shape. Subsequently, the device is immersed and left overnight into the n-Wet 410 solution to modify the entire surface of the PDMS to a hydrophilic state (figure 1a-viii). Finally, the top surface of the device is microcontact stamped with a thin layer of PDMS prepolymer and cross linked in order to make only that surface hydrophobic (figure 1a-ix).

**Experimental procedure.** During experiments 0.01 mL sample solution containing bacteria is dispensed on the surface (figure 1b-ii). Next, a glass slide is used as a squeegee to move the liquid around on the surface. The sample liquid automatically enters and compartmentalize into the nanowells as it is moved around (figure 1b-iii). The device is then sealed with a glass slide coated using a commercial hydrophobic surface modification agent (Aquapel®) and imaged under a microscope to measure the fluorescence intensity of the oxygen quenching fluorophore. A confocal microscope was used throughout the experiments to capture images, whereby the *E.coli* is located on a specific plane, and thus, the fluorescence of