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G. Comina,^a A. Suska^a and D. Filippini*^a

3D printed device for quantitative enzymatic detection using cell phones

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A disposable device for quantitative enzymatic detection capable of coupling illumination and imaging readout from cell phones is demonstrated. The device integrates a calibration range for glutamate detection, utilizes the phone screen as a light source, and provides the necessary actuation for autonomous operation. Custom made optics required to couple to the cell phone camera is accomplished using affordable stereolithography (SLA) 3D printers. The described method do not involve polishing, requires only two steps from design to implementation, and can be locally applied to 3D printed lab-on-a-chip (LOC) prototypes, using the same materials. Optical finishing and dimensional variability within 2% were achieved, supporting entirely arbitrary geometries for elements larger than 400 µm in radius. Representative fabrication times and costs were 20 min and \$0.50 USD/prototype.

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Reproducibility of the coupling lens

Reproducibility of the coupling lens was characterized by sextuplicates and evaluated via the resulting magnification, which summarizes in a single parameter the printer reproducibility, the final curvature, and the influence of the coating procedure. Finished lenses were imaged under identical conditions while resting on millimeter paper. A sector of the millimeter paper without lens was imaged together with the lens in all cases.

The lens magnification was evaluated as the ratio of the length in pixels of a 6 mm distance without lens (I_2) , and the same 6 mm as magnified at the center of the lens (I_1) (Fig. S1). The resulting average value and corresponding error for a 95% confidence interval represent a 2% variation within the reported batch.





Fig. S1. Six lenses printed with the Form+1 platform and coated with 10 μ L of Form+1 Clear version 02 resin. The variability was assessed as the ratio in pixels in the lens center (l_1) and outside the printout (l_2) for a 6 mm-long segment. The average ratio measured for these six lenses has a variability of 2.0% for a 95% confidence interval.

+ Footnotes relating to the title and/or authors should appear here.

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^{a.} Optical Devices Laboratory, IFM, Linköping University 58183 Linköping (Sweden), E-mail: danfi@ifm.liu.se.

Printer resolution

Fill factors reported in Fig. S2a were determined using the .png slices created by the Miicraft[®] Suite. Images from the respective lens designs were zoomed to single pixel magnification in Adobe Photoshop CS4, where the white background was masked to the edge of the designed lens diameter. The remaining pixels were colored blue, and the resulting images were exported to Image J 1.440 (NIH, USA), where the black and white pixels were counted. The resulting fill factor was computed as the fraction of black pixels for each lens size.

Uncoated 3D printed elements cannot act as lenses since the geometry is made of the stacking of multiple flat surfaces. Once coated the jagged geometry is filled, then producing smooth surface curvatures and functional lenses.

In the case of the 3 mm radius lens, a 3x magnification (Fig. S2a) can be directly observed as a 4x1 mm cell from the background millimeter-scaled paper that fills the lens area. For r < 1 mm a ring-shaped 100 μ m deep ditch can be used to minimize the meniscus at the edge of the lens, and to maintain the designed curvature for all sizes within a 90° field of view range (Fig. S2b).

The effect of the coating on the designed curvature (Fig. S2b) was characterized with and optical method. The lenses were positioned besides a mirror at a 45° degree angle with respect to the surface and imaged at fixed magnification with a Olympus SZ60 stereo zoom microscope (Olympus Corporation, Shinjuku, Tokyo, Japan), fitted with a Canon EOS 500D DSLR camera (15 MP, APS-C cmos sensor, Canon Inc., Ōta, Tokyo, Japan).

The curvature of the lenses were characterized before and after coating via image processing using Adobe Photoshop CS4 (Adobe Systems Inc., San Jose, California, USA), involving contrast enhancement and thresholding to separate the lens profile from the background. Subsequently, color assignment and multilayer merging were used to render the profiles before and after coating (Fig. S1b, orange and cream colors, respectively). The final result closely matched the designed geometry within a 90° field-of-view for all lenses. As anticipated, the error is greater for smaller lenses, which are more roughly defined by the 3D printout (Fig. S2a). However, the error was acceptable even for the 400 μ m radius lens, which represents the lower size limit for these printers.

Fig. S2c and d illustrate arbitrary geometries of optical components that include aspherical, conical, elliptic, and faceted bodies at the critical size for the printers used, as well as a cylindrical light guide illuminated by a laser beam (Fig. S2d). The alternatives to produce such configurations are more expensive, less versatile, demand specialized skills, or demand infrastructure that is orders of magnitude more expensive.



Fig. S2a) CAD designs of semi-spherical lenses of radius r = 3, 2, 1, 0.8 and 0.4 mm, and their conversion to 3D printer resolution, where each pixel is represented by 50 μ m side cubes; photographs of the actual printed lenses without coating and after coating, on millimeter-scaled paper. b) Lens curvature before and after coating for r = 3, 2, 0.8 and 0.4 mm. c) Aspherical, faceted conical, cylindrical conical, elliptical, and arbitrary geometry within a 1 mm radius, before and after coating. d) Cylindrical light guide before and after coating, showing the light path of a red laser beam.

Alternative coatings

Optical elements illustrated in Fig. S3a,b were printed with the Miicraft platform, and coated with the Miicraft transparent resin. This material has a refractive index of n = 1.35, as estimated by measuring the refraction of a red laser beam (635 nm pointer) on a 3D printed block (5 mm x 35 mm x 25 mm). The estimated refractive index for the Form1+ resin was n = 1.53. Both types of printouts are also compatible with coatings of the NOA 68 (Norland Products Inc., Cranbury, New Jersey, USA) photo curable polymer having a refractive index of 1.56, and with PDMS. (Fig. S3).



Fig. S3. Lenses printed with a Miicraft SLA 3D printer and coated with 10 μL of Miicraft resin and PDMS (upper images). Lenses printed with a Forms1+ SLA 3D printer and coated with 10 μL of NOA 68 and Form1+ resin (lower images).

Assay linear range and variability

The glutamate bioassay linear range was established by complementary measurements at six different glutamate concentrations: 12.5, 25, 50, 100, 150 and 200 μ g/dL (Fig. S4a). For the depicted analysis window of 10s the cumulative response was computed and illustrated in Fig. S4b. From this cumulative response, the response time vs. glutamate concentration was computed (Fig. S4c), and linear fitting to the result corresponds to a regression coefficient of 0.996.

The assay average variability was evaluated for triplicates at 50, 100 and 200 μ g/dL of glutamate (insert in Fig. S4b), which corresponds to ±266 ms (±5.31%). Error bars in Fig. S4c indicate the assay variability, whereas the detection error is within the width of the blue line.



Fig. S4a) Collection of ROIs corresponding to 6 different glutamate concentrations: 12.5, 25, 50, 100, 150, 200 μ g/dL, and their green channel average intensity. Measurements were performed in devices with multiple replicas of the detection sector and mixer geometry (inset). b) Cumulative intensity shown in the 35 to 45 s interval, used to quantify the time response. In the inset, the repeatability at three different concentrations was tested in triplicate, resulting in ±266 ms average variability or ±5.31%. c) Linear range corresponding to a regression coefficient of 0.996.