## A low-cost smartphone-based device for point-of-care ovulation testing

## Authors

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## **Supplementary Information**



Node MCU + ESP12E- Motor Shield

**Figure S1. Circuit diagram of the electrical enclosure of the optical system.** Wireless communication between the smartphone and the optical attachment was achieved by configuring the Single-board microcontroller (NodeMCU) as an http web server. The system includes a DC motor for automation, a LED for illumination, a 9 V battery for power source, and a switch for control purposes. All components are connected to the NodeMCU.



Figure S2. Image of micrometer scale and 1951 US Air Force (USAF) resolution test chart recorded with the smartphone system. (A) The spacing between the divisions of the stage micrometer (Omax, B00FG89F0M) is 10  $\mu$ m. The dimensions of the image shown here are 642 × 642 pixels. One micron is represented by 0.226 pixels. (B) Resolution of the reported smartphone-based optical imaging system was 7 $\mu$ m.



**Figure S3. Smartphone application user interface.** These figures show the process flow of the android application developed for image processing and ovulation detection. (A) The smartphone

application can be selected on the home screen of the smartphone. The smartphone application icon is shown within the red box in this figure. (B) The home screen of the application shows different options that can be accessed. The schematic has been color-coded to show the different possibilities. (i) Red color option leads to initialization of sample testing; (ii) blue color option leads to test result history; and (iii) green color option leads to a calendar to track ovulation cycle. (C) Time remaining for completion of the test is first displayed followed by the result.



Figure S4. Drying time for human saliva samples. Different volumes of saliva samples were used on the developed microfluidic device and on a simple glass slide to test the drying time at room temperature. The slopes of the regression lines for the microfluidic device and the glass slide groups were 0.01 and 0.52 with  $R^2$  values of 0.94 and 0.06, respectively. Each data point represents the mean value (n=3) while the error bars represent the standard error of mean.



Figure S5. Confusion matrices for the test sets using artificial saliva and human saliva samples. (A) The system accuracy when artificial saliva samples (n=200) were tested was 90%. (B) The system accuracy when human saliva samples (n=200) were tested was 99.5%. Prior to testing, the samples were classified into ovulating and non-ovulating based on the urine test results.

| Materials cost      |            |             |  |  |  |
|---------------------|------------|-------------|--|--|--|
|                     | Cost (USD) | Total (USD) |  |  |  |
| Hardware attachment |            | 13.58       |  |  |  |
| Lenses (both)       | 1.73       |             |  |  |  |
| PLA                 | 1.52       |             |  |  |  |
| LED                 | 0.1        |             |  |  |  |
| Battery             | 1.25       |             |  |  |  |
| Switches and wires  | 0.7        |             |  |  |  |
| Node MCU            | 3.47       |             |  |  |  |
| DC motor            | 3.22       |             |  |  |  |
| Linear rods         | 1.59       |             |  |  |  |
|                     |            |             |  |  |  |
| Microchip           |            | 0.33        |  |  |  |
| Glass slide         | 0.19       |             |  |  |  |
| PMMA                | 0.09       |             |  |  |  |
| DSA                 | 0.006      |             |  |  |  |
| PLA                 | 0.04       |             |  |  |  |
|                     |            |             |  |  |  |
| Total               |            | 13.91       |  |  |  |

 Table S1. Estimated material costs of the hardware. Material costs for all the elements used

 in the fabrication of the optical attachment and the microfluidic chip.

| Artificial saliva<br>samples | Samsung<br>Galaxy 5 | Xiaomi<br>Redmi Note<br>4 | OnePlus 5T | LG G6      | Moto X     |
|------------------------------|---------------------|---------------------------|------------|------------|------------|
| Sample 1                     | Ferning             | Ferning                   | Ferning    | Ferning    | Ferning    |
| Sample 2                     | Ferning             | Ferning                   | Ferning    | Ferning    | Ferning    |
| Sample 3                     | Ferning             | Ferning                   | Ferning    | Ferning    | Ferning    |
| Sample 4                     | Ferning             | Ferning                   | Ferning    | Ferning    | Ferning    |
| Sample 5                     | Ferning             | Ferning                   | Ferning    | Ferning    | Ferning    |
| Sample 6                     | Ferning             | Ferning                   | Ferning    | Ferning    | Ferning    |
| Sample 7                     | Ferning             | Ferning                   | Ferning    | Ferning    | Ferning    |
| Sample 8                     | Ferning             | Ferning                   | Ferning    | Ferning    | Ferning    |
| Sample 9                     | Ferning             | Ferning                   | Ferning    | Ferning    | Ferning    |
| Sample 10                    | Ferning             | Ferning                   | Ferning    | Ferning    | Ferning    |
| Sample 11                    | No Ferning          | No Ferning                | No Ferning | No Ferning | No Ferning |
| Sample 12                    | No Ferning          | No Ferning                | No Ferning | No Ferning | No Ferning |
| Sample 13                    | No Ferning          | No Ferning                | No Ferning | No Ferning | No Ferning |
| Sample 14                    | No Ferning          | No Ferning                | No Ferning | No Ferning | No Ferning |
| Sample 15                    | No Ferning          | No Ferning                | No Ferning | No Ferning | No Ferning |

**Table S2. Software performance when different smartphones used**. Artificial saliva samples were diluted to create samples with and without fern structures. All samples were imaged an analyzed by each smartphone individually.