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The geek and the chemist: antioxidant capacity measurements by DPPH assay in beverages using open source tools, consumer electronics and 3D printing

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Microcontrollers and single-board computers are widespread tools for innovative educational labs, for prototyping and for accomplish everyday tasks by expert users. Moreover, these modules are opening new exciting possibilities in the area of biological and chemical assays. In this study a Raspberry Pi computer assembled with 3D printed parts and inexpensive opto-electronic components were employed to analyse the antioxidant capacity of several bottled tea performing diphenylpicryl-hydrazyl (DPPH) tests. A dedicated python software allowed the execution of the analysis controlling the device through a small LCD touch screen or remotely through secure connections with other devices. The rRaspberry Pi-based measurements were compared with a research-grade spectrophotometer showing excellent correlation (R2=0.9996) and no significant differences ($p < 0.05$) in the range of measured values. We strongly believe that this approach could support diagnostics progress in resource-poor countries and open new opportunities in research and education.

1. Introduction

The recent advancements in the field of consumer electronics are expanding the employment of smart, low cost devices for specific tasks beyond the common everyday use. The widespread availability of smartphones and the increasing number of universal electronic modules are opening new exciting possibilities in the area of biological and chemical assays.

Smartphones have been recently proposed as a core element for the creation of innovative platforms that will have a strong impact on science, engineering and diagnostics, partially bringing the functions of advanced laboratory instruments to field conditions, enabling new telemedicine, point-of-care and mobile diagnostics possibilities, having thus implication also on democratization of science, research and education [1,2].

Thanks to the embedded high-end component performances, smartphone-based devices have been recently proposed as diagnostic tools for microscopy [3–5], blood analysis and cytometry [6–8], detection of nucleic acids [9], microorganisms and pathogens [10–13] sensing of allergens [14], urine [15–18], sweat, and saliva parameters monitoring [19], and solar UVA radiation measurement [20,21].

The potential success for these smartphone-based platforms is mainly based on some common features: i) the type and the quality of the embedded components and sensors such as accelerometers, IR sensors, gyroscopes, barometers, magnetometers: in particular, the opto-electronic units based on CMOS or CCD sensors that are in mobile cameras have currently reached a performance level that allows their use in analytical applications. Basically, every assay that works in the visible range which is based on absorbance, reflectance or fluorescence reading could be adapted to a smartphone-based format; ii) the power of processors and the strong, already available infrastructure for connectivity offer noteworthy computational power and data sharing capabilities to users; iii) the opportunity to easily benefit from cloud-based services and software (apps) with improved usability and more customization compared to the traditional analytical approaches.

The utilization of smart devices like smartphones, tablets and wearables (i.e. google glass and smart-watches) for analytical applications might pose some potential drawbacks and limitations. In some cases, a significant limitation could arise from the “smart nature“ of these objects as they are currently conceived. Indeed, in order to offer a ready-to-use tool focused on the optimization of user experience for consumer applications, these devices are closed pieces of hardware that offer poor customization capabilities. Moreover, every apparatus has different hardware components and many aspects of the configuration as well as many developed software parts are not disclosed in details. This scenario could represent an obstacle to the implementation of analytical applications using commercial smart devices. In particular, assay developers will be forced to face the needs for implementation of tests using a wide range of devices making the harmonization of methods very hard. On the other hand, the assay developers risk resulting totally dependent on the choices of smart device manufacturers that control the consumer market with rapid changes in hardware and software guidelines, often driven by marketing reasons. This scenario will not probably pose an insuperable threat for the diffusion of analytical approaches for smart devices but, in some specific cases, could be limiting.

A promising approach, particularly in the field of research, prototyping and educational sector, seems to be the fast growing area of the universal electronic modules [22]. These electronic devices are units that can easily be adapted to specific tasks by means of open architectures, modular concepts and programming through high-level languages. These devices are often “open source” systems, quite inexpensive and make use of a wide range of sensors, actuators and expansion modules available for customization. The modularity, along with powerful open-source software libraries, makes them the perfect tools for research, trial and error learning and, more in general, cross-contamination of science fields.

The key aspect for the employment of these platforms in biological and chemical analysis is represented by some specific features: i) the possibility of open and flexible (digital and analogical) input/output signal routing that enable to connect modules such as motors, sensors, LEDs, ecc; ii) the easy connectivity to computers and other devices (USB, ethernet, wi-fi, bluetooth) that facilitates the programming and the integration of the devices with other piece of consumer electronics; iii) the active communities of open-source oriented programmers that allow rapid problem solving and shortening of the learning curve for nonprofessional users.

Two main architectures seem to be attractive in the area of analytical tests. One is represented by microcontrollers based on printed circuit board (PCB) like Arduino and its derivative like Trinket or Propeller mini. These devices are usually programmed using dedicated environment interfaces that give complete control to their hardware but have limited programming capabilities.

The second type of architecture is represented by the single-board computer which relys on a real and complete operative system (OS) and offers more flexibility and power in terms of programming solutions. Usually the OS (often an optimized version of linux releases) is flashed on an SD card and offers several programming tools and high-level programming languages such as C, C++, java, python. Examples of single-board computers are Raspberry Pi, BeagleBone, Intel Galileo. The results presented herein are focused on applications of Raspberry Pi to chemistry analysis.

Raspberry Pi is a low cost credit-card sized computer developed by the Raspberry Pi foundation with the main aim to advance the education of adults and children in the field of computers, computer science and related subject.

The Raspberry Pi is currently available in many models that share the same system on chip, a full HD multimedia applications processor for advanced mobile and embedded applications. This tool is one of the most diffused and supported single-board computers; a wide range of Rasperry Pi-compatible devices and expansion board are available. Interestingly, two dedicated camera modules (a standard one and an IR sensitive version) are currently offered.

The Rasperry Pi camera can be controlled directly by launching line driven applications via linux terminal. Alternatively a package of pure Python application programming interface is available. The Raspberry Pi camera is particularly interesting for chemical and biological analysis applications because a vast range of low-level parameters can be controlled by the user. This can help to overcome some limitations associated to consumer cameras (e.g. smartphones) for the acquisition of analytical data: the access to the image treatment algorithms, the possibility to switch off the automatic adjustment of white balance and control the exposure mode, the access to raw data and to direct pixel intensity values are some of the operations that can be easily achieved by using Raspberry Pi camera that can improve the quality of the analytical data. Moreover, the availability of powerful software tools and libraries makes Raspberry Pi an interesting tool for diagnostic research and prototyping.

On the other hand, electrochemical and optical bio/chemosensoristic devices require constant improvement on key aspects like integration, portability, cheapness, simplification of experimental protocols, and development of efficient high-throughput approaches in electrochemical and optical bio/chemosensoristic devices.[23]. Beside this well-established topics related to portable diagnostics, new ones are rapidly gaining ground: connectivity for real time remote access and big data analysis, design of tools that meet the needs of diagnostics in resource-poor settings through customization of consumer electronics components [24], applications of open-source 3D printing and microcontrollers running on free software to enable the development of powerful, low-cost and highly customizable research tools for scientists, engineers, and lab personnel [25].

In order to test and demonstrate the feasibility of Raspberry Pi-based devices for diagnostics-oriented applications, here a common assay for the evaluation of scavenging activity of antioxidant molecules in food based on diphenylpicryl-hydrazyl (DPPH) [26] was carried out using the Raspberry Pi-based devices and the results were compared with a conventional laboratory-based approach.

2. Materials and methods

2.1. *Raspberry Pi settings*

The Advanced RISC machine based computer Raspberry Pi (version B+) was employed as computing unit and its camera module was employed as sensor for all the experiments.

The device was installed with the standard linux-based Raspbian operating system (kernel 3.15.3+) and the experiments were carried out connecting a 320 x 240 2.8’’ LCD TFT touch screen (Adafruit

Industries, New York City, USA). Alternatively, some measurements were carried out connecting common laptops to the Raspberry Pi-based device through the standard SSH protocol.

The software employed during the experiments was developed entirely using Python (ver. 2.7.3) and the camera module was controlled using the picamera package, a pure python interface released under BSD licence. A simple graphical user interface (GUI) for the analysis management was created using the python standard GUI package TkInter.

The software module that manages the acquisition of the images was managed in order to disable the automatic selection of the exposure and the application of any kind of effects. Moreover, the auto-white balance mode was disabled and a warm up time (5 seconds) for the camera circuitry was declared.

2.2. Device assembling

A simple and inexpensive optical set-up was used for the absorbance measurements. The set up was realized in a modular way in order to add and/or replace optical components at any time without changing the geometry of the entire device. A 5 mm diameter round green LED was used as a light source, while a diffuser was placed between the light source and the cuvette holder in order to uniform the light signal. A 50 mm focal length, 25 mm diameter uncoated plano convex lens has been placed between the cuvette and the camera. The camera, a 5Mpx CMOS sensor, was connected to the Raspberry Pi board and captured images were used by the software to calculate the absorbance values. All the components, placed inside a 90x62x60 mm case, as shown in Fig. 1, have been designed and fabricated *in-house* by means of a 3D printer Sharebot Next Generation (Sharebot, Nibionno, LC, Italy) by using the fused deposition modeling technique of a poly(lactic acid), a plastic material made of vegetable fibers.

2.3. Sample preparation

The samples for the preliminary evaluation of the device were obtained by dilutions of the food coloring “amaranth” (IUPAC name: Trisodium (4E)-3-oxo-4-[(4-sulfonato-1-naphthyl)hydrazono]naphthalene-2,7-disulfonate) in double distilled water. The absorbance was measured by using conventional spectrophotometers (Perkin Elmer Lambda 20 bio; Agilent Technologies Cary 60). All the samples were prepared in order to have absorbance values ranging from 0.1 to 1.0.

The samples employed for the study were bottled teas purchased in local stores located in Parma (Italy). All the samples were diluted 1:10 with double distilled water and used within 1 hour for the DPPH assay. No further sample treatment procedures were carried out before the analysis.

2.4. DPPH assay

The radical-scavenging activity of bottled teas was evaluated using the DPPH assay. Aliquots of each tea dilution (100 ml) were added to 3.0 ml of DPPH solution (76×10^{-6} mol L^{-1}) in methanol. The change in absorbance was measured using the Raspberry pi-based device camera module and the developed software. The results were compared to data obtained using a conventional spectrophotometer (Perkin Elmer Lambda 20 bio) monitoring the absorbance at 517 nm. Disposable polystyrene cuvettes were employed for both methods. The same samples were read sequentially employing the two instruments.

Results are expressed as percentage of inhibition, according to the expression $[(A_0 - A_s)/A_0] \times 100$, where A_0 is the initial DPPH absorbance (3.0 ml of DPPH solution + 100 ml of double distilled water) and A_s is the absorbance of sample added to DPPH solution after 30 minutes. The correctness of the reaction time was also controlled performing kinetic analysis [26].

2.5. Absorbance calculation

The absorbance calculation using Raspberry Pi was obtained by image processing. The images were acquired at maximum resolution of 2592 x 1944 pixels, and then saved on the microSD card during the assay for further processing. The same region of interest (ROI) of 121 x 103 pixels was cropped from the original images and the RGB values for each pixel were stored in a matrix by the python script.

The stored data were used for the calculation of mean RGB values. The absorbance (A) was calculated from green (G) channel values considered as intensity, according to:

$$A = \log \frac{I_0}{I} \qquad (1)$$

were I_0 is the input light intensity of the LED source and I is the output intensity, that is the light measured just after the sample. The absorbance calculated for the solvent (methanol) was subtracted to each measured sample of tea. The whole process for the elaboration of 39 samples and for absorbance calculation required approximately 6 minutes. Results were saved as plain text file.

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1. Introduction

The recent advancements in the field of consumer electronics are dramatically expanding the employment of smart, low cost devices for specific tasks beyond the common everyday use.

The widespread availability of smartphones and the increasing number of universal electronic modules are opening new exciting possibilities in the area of biological and chemical assays.

Smartphones are recently proposed as core for the creation of innovative platforms that will have a strong impact on science, engineering and diagnostics, partially bringing the functions of advanced laboratory instruments to field conditions, enabling new telemedicine, Point-of-care (POC) and mobile diagnostics possibilities, having thus implication also on democratization of science, research and education [1,2].

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The employment of smart devices like smartphones, tablets and wearable devices (like the google glass and the smart-watches) for analytical applications might pose also some potential drawbacks and limitations. In some cases, a significant limitation could arise from the “smart nature“ of these objects as they are currently conceived. Indeed, in order to offer a ready-to-use device focused on the optimization of user experience for consumer applications, these devices are closed pieces of hardware that offer poor customization capabilities. Moreover, every device has different hardware components and specifications and many aspects of these configurations, as well as many developed software parts, are not disclosed in details. This scenario should represent an obstacle to the implementation of analytical applications using commercial smart devices. In particular, assay developers will be forced to face the needs for implementation of tests using a wide range of devices making the harmonization of methods very hard. On the other hand, the assay developers risk to result totally dependent by the choices of smart device manufacturers, that refer to consumer markets with rapid changes in hardware and software guidelines, often driven by marketing reasons. This scenario will not probably pose an insuperable threat for the diffusion of analytical approaches for smart devices but, in some specific cases, could be constraining.

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The results presented herein are focused on applications of Raspberry Pi to chemistry analysis.

Raspberry pi is a low cost (around 30€) credit-card sized computer developed by the Raspberry Pi foundation with the main aim to advance the education of adults and children in the field of computers, computer science and related subject.

The Raspberry Pi is currently available in five models that share the same system on chip (SoC), the Broadcom BCM2835, a full HD multimedia applications processor for advanced mobile and embedded applications. This tool is one of the most diffused and supported single-board computers; a wide range of Rasperry Pi-compatible devices and expansion board are available. Interestingly, two dedicated camera modules (a standard one and an IR sensitive version) are currently offered.

The Rasperry Pi camera can be controlled directly launching line driven applications *via* linux terminal. Alternatively a package of pure Python application programming interface (API) (under the name PiCamera) is available. The Raspberry Pi camera is particularly interesting for chemical and biological analysis applications because a vast range of low-level parameters can be controlled by the user. This can help to overcome some limitations associated to consumer cameras (e.g. smartphones) for the acquisition of analytical data: the access to the image treatment algorithms, the possibility to switch off the automatic adjustment of white balance and control the exposure mode, the access to raw data and to direct pixel intensity values are some of the operations that can be easily achieved using Raspberry Pi camera that can improve the quality of the analytical data. Moreover, the availability of powerful software tools and libraries makes Raspberry Pi an interesting tool for diagnostic research and prototyping.

In order to test the feasibility of Raspberry Pi-based devices for diagnostics-oriented applications, a common assay for the evaluation of scavenging activity of antioxidant molecules in food based on diphenylpicryl-hydrazyl (DPPH) [23] was carried out using the Raspberry Pi-based devices and the results were compared with a conventional laboratory-based approach.

2. Materials and methods

2.1. *Raspberry Pi settings*

The Advanced RISC machine (ARM) based computer Raspberry Pi (version B+) was employed as computing unit and its camera module was employed as sensor for all the experiments.

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2.2. Device assembling

A simple and inexpensive optical set-up was used for the absorbance measurements. The set up was realized in a modular way in order to add and/or replace optical components at any time without changing the geometry of the entire device. A 5 mm diameter round green LED was used as a light source, while a diffuser was placed between the light source and the cuvette holder in order to uniform the light signal. A 50 mm focal length, 25 mm diameter uncoated plano convex lens has been placed between the cuvette and the camera. The camera, a 5Mpx CMOS sensor, was connected to the Raspberry pi board and captured images used by the software to calculate the absorbance values. All the components were placed inside a 90x62x60 mm case, as reported in Fig. 1c.

All the components have been designed and fabricated *in-house* by means of a 3D printer Sharebot Next Generation (Sharebot, Nibionno, LC, Italy). All the components were printed using the fused deposition modeling (FDM) technique and poly(lactic acid) (PLA), a plastic material made of vegetable fibers, was used.

2.3. Sample preparation

The samples for the preliminary evaluation of the device were obtained by dilutions of the food coloring "amaranth" (IUPAC name: Trisodium (4E)-3-oxo-4-[(4-sulfonato-1-naphthyl)hydrazono]naphthalene-2,7-disulfonate) in double distilled water. The absorbance was measured using a conventional spectrophotometer (Perkin Elmer Lambda 20 bio). All the samples were prepared in order to have absorbance values ranging from 0.1 to 1.0.

The samples employed for the study were bottled tea purchased in local stores located in Parma (Italy). All the samples were diluted 1:10 with double distilled water and used within 1 hour for the DPPH assay. No further sample treatment procedures were carried out before the analysis.

2.4. DPPH assay

The radical-scavenging activity of bottled teas was evaluated using the DPPH assay. Aliquots of each tea dilution (100 □l) were added to 3.0 ml of DPPH solution (76 x 10^{-6} mol L^{-1}) in methanol. The change in absorbance was measured using the Raspberry pi-based device camera module and the developed software. The results were compared to data obtained using a conventional spectrophotometer (Perkin Elmer Lambda 20 bio) monitoring the absorbance at 517 nm. Disposable polystyrene cuvettes were employed for both methods. The same samples were read sequentially employing the two instruments.

Results were expressed as percentage of inhibition, according to the expression $[(A_0 - A_s)/A_0] \times$

100, where A_0 is the initial DPPH absorbance (3.0 ml of DPPH solution + 100 ml of double distilled water) and A_s is the absorbance of sample added to DPPH solution after 30 minutes. The correctness of the reaction time was also controlled performing kinetic analysis (Pyrzynska and Pękal, 2013).

2.5. Absorbance calculation

The absorbance calculation using Raspberry Pi was based on image processing. The images were acquired at maximum resolution of 2592 x 1944 pixels, and then saved on the microSD card during the assay for further processing. The same region of interest (ROI) of 121 x 103 pixels was cropped from the original images and the RGB values for each pixel were stored in a matrix by the python script.

The stored data were used for the calculation of mean RGB values. The absorbance (A) were calculated from green (G) channel values treated as intensity, according to:

$$A = \log\frac{I_0}{I} \qquad (1)$$

were I_0 is the intensity of the LED source and I is the intensity of samples. The absorbance calculated for the solvent (methanol) was subtracted to each measured sample of tea.

The whole process for the elaboration of 39 samples and for absorbance calculation required approximately 6 minutes. Results were saved as plain text file.

3. Results and discussion

3.1. Set-up optimization

The Raspberry Pi boards were developed with the major intent to support the teaching of basic computer science in school. However, these devices have a strong potential for analytical applications thanks to the low cost and the open hardware architecture.

A main reason for the great versatility of Raspberry Pi is the hardware connectivity: the possibility to connect and control a wide spectrum of devices, sensors and light sources make this tool particularly suitable also for prototyping of small readers for biological and chemical assays.

In order to evaluate the capability of Raspberry Pi and compatible consumer electronics as components for an analytical device, a simple set-up was designed and realized by means of 3D printing (Fig 1). In particular, the camera based on the OmniVision OV5647 CMOS image sensor was used as detector and a green LED was used as light source. Both the components were directly connected to Raspberry Pi and controlled using python libraries: the camera was mounted on the specific socket of the board and was controlled through the python library “picamera” and the LED was connected to the appropriate General Purpose Input/Output (GPIO) pins and controlled with the “wiringpi2” library. The other components of the device were a lens placed in font of the camera, a cuvette holder and a plastic diffuser placed in front of the LED source. All the components were mounted on a 3D-printed plastic guide-rail that allowed the variation of relative distances for each component inside the case.

Raspberry Pi does not provide an Digital to Analog Converter (DAC) interface for generating analog voltage signals in order to control the variation of the LED intensity; however, this function was achieved *via* pulse-width modulation (PWM) digital control, varying the duty cycle between 0 to 1024 values. In order to evaluate the capability of the camera to detect signal variations, different LED’s intensities were imaged and analysed (Fig. 2). According to the results, the LED’s intensity can be easily set to green (G) intensities that span a suitable range of the possible 8-bit values. For the subsequent experiments the PWM were set to 800, which corresponded to a G value of 165.18 ± 0.09 (measure in triplicate). All the measurements were carried out using one of the two dedicated GPIO pins offering PWM under direct control of dedicated portions of hardware. Despite other GPIO pins provide the PWM functionality, they are totally software-driven and rely on CPU processes, which is not powerful enough to assure stable values. This option was also considered and tested, but resulted unreliable under heavy CPU load, compared to the hardware PWM option.

3.2. Data Elaboration

Raspberry Pi offers a wide spectrum of parameters control for the camera module. In particular, the capability of disabling automatic and software-driven parameters and control the parameters value resulted particularly useful for the optimization of the analysis. In fact, using the default camera settings, ambiguous results and lack of reproducibility were noticed during the preliminary tests (data not shown).

The best results were obtained by disabling the automatic exposure mode, the automatic white balance mode and setting the exposure speed to a fixed value. The subsequent experiments were carried out using the same settings.

All the operations on the Raspberry Pi-based device were driven by dedicated python scripts that control and execute all the steps of the analysis. The logical scheme for the DPPH assay is described in Fig. 3a. The reading of samples was controlled by an acquisition module that acquire the image at full resolution (2592 x 1944) from the camera with optimized parameters, crop a ROI (121 x 103) and calculate the mean values for RGB data (Fig. 3b). The same calculation module and the same parameter settings were employed for all the analysis. Other modules execute the absorbance calculation for each sample and the calculations for the % inhibition for the DPPH assay. When the graphical interface is employed, a module provides the results as histogram graph.

3.3. Set-up calibration

In order to investigate the capability to detect the variation of concentration of a given molecule, several dilutions of the food dye amaranth, which has an absorbance maximum close to DPPH, were analysed. The same samples were also read using a conventional spectrophotometer as reference method. In order to test also the repeatability over the selected range of concentrations, five measurements for each point were performed. The results, reported in Fig. 4, showed an excellent correlation between measured values and dye concentrations. Moreover the Raspberry Pi-based device showed CV% values (0.25 ÷ 1.05) that resulted comparable with the data obtained using a conventional spectrophotometer (which exhibited values of 0.11 ÷ 0.72).

Since the assembled device exhibited the capability to measure samples with different concentrations and showed good performances compared with a reference instrument, a DPPH analysis using real samples was carried out.

3.4. DPPH assay

The Raspberry Pi-based device was evaluated performing a diagnostic test using real samples. The DPPH assay was chosen for this purpose because it is a very popular method in food chemistry for evaluating the ability of compounds to act as free radical scavengers or hydrogen donors, evaluating the antioxidant capacity of foods. The DPPH radical is a commercially available long-lived organic nitrogen that does not have to be generated before the assay. This molecule has a deep purple colour that turns to yellow when is mixed with an antioxidant/reducing compound. The relative reducing ability of antioxidants can be evaluated by monitoring the decrease of its absorbance at 515–528 nm as consequence of the formation of the corresponding hydrazine $DPPH_2$, that yields a yellow solution [23].

The DPPH analysis was performed on 13 commercial bottled tea purchased in local stores located in Parma (Italy). All the samples were analysed without treatment excepted for a 1:10 dilution in double distilled water. The samples were analysed in triplicate using the Raspberry pi-based device and compared with values obtained using a conventional spectrophotometer as reference method. The same samples were read sequentially employing the two instruments and the reading events for each sample occurred almost contextually (the time-lag between the reading performed with the two methods was approximately 30 seconds). The results are showed in Table 1 and the correlation between the two sets of measures in Fig 5. The correlation between the two measurement methods resulted very high (R^2=0.9996) and no significant differences were observed ($p < 0.05$), at least in the range of measured values.

4. Conclusion

In summary, a Raspberry Pi computer along with its CMOS camera assembled with 3D printed parts and inexpensive opto-electronic components were successfully employed to build a cheap reader for absorbance analysis. In order to evaluate the Raspberry Pi-based device for diagnostics-oriented applications, a DPPH-based assay was carried out in triplicate on several commercial bottled tea and the results were compared with the data obtained using a research-grade spectrophotometer. No statistically significant differences were observed between the two instruments for the tested samples.

We strongly believe that these kind of devices in association with advanced smart software (APPs) and a proper web community could support diagnostics progress in resource-poor countries and strongly foster the innovation in research and learning processes for science and diagnostics.

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References

[1] A. Ozcan, Mobile phones democratize and cultivate next-generation imaging, diagnostics and measurement tools., Lab Chip. (2014) 3187–3194. doi:10.1039/c4lc00010b.

[2] P. Preechaburana, A. Suska, D. Filippini, Biosensing with cell phones, Trends Biotechnol. 32 (2014) 351–355. doi:10.1016/j.tibtech.2014.03.007.

[3] D.N. Breslauer, R.N. Maamari, N. a. Switz, W. a. Lam, D. a. Fletcher, Mobile phone based clinical microscopy for global health applications, PLoS One. 4 (2009) 1–7. doi:10.1371/journal.pone.0006320.

[4] Z.J. Smith, K. Chu, A.R. Espenson, M. Rahimzadeh, A. Gryshuk, M. Molinaro, D.M.

Dwyre, S. Lane, D. Matthews, S. Wachsmann-Hogiu, Cell-phone-based platform for biomedical device development and education applications, PLoS One. 6 (2011). doi:10.1371/journal.pone.0017150.

[5] I. Navruz, A.F. Coskun, J. Wong, S. Mohammad, D. Tseng, R. Nagi, S. Phillips, A. Ozcan, Smart-phone based computational microscopy using multi-frame contact imaging on a fiber-optic array., Lab Chip. 13 (2013) 4015–23. doi:10.1039/c3lc50589h.

[6] D.J. You, T.S. Park, J.Y. Yoon, Cell-phone-based measurement of TSH using Mie scatter optimized lateral flow assays, Biosens. Bioelectron. 40 (2013) 180–185. doi:10.1016/j.bios.2012.07.014.

[7] H. Zhu, S. Mavandadi, A.F. Coskun, O. Yaglidere, A. Ozcan, Optofluidic fluorescent imaging cytometry on a cell phone, Anal. Chem. 83 (2011) 6641–6647. doi:10.1021/ac201587a.

[8] H. Zhu, I. Sencan, J. Wong, S. Dimitrov, D. Tseng, K. Nagashima, A. Ozcan, Cost-effective and rapid blood analysis on a cell-phone., Lab Chip. 13 (2013) 1282–8. doi:10.1039/c3lc41408f.

[9] D. Lee, W.P. Chou, S.H. Yeh, P.J. Chen, P.H. Chen, DNA detection using commercial mobile phones, Biosens. Bioelectron. 26 (2011) 4349–4354. doi:10.1016/j.bios.2011.04.036.

[10] H. Zhu, U. Sikora, A. Ozcan, Quantum dot enabled detection of Escherichia coli using a cell-phone, Analyst. 137 (2012) 2541. doi:10.1039/c2an35071h.

[11] L.P. Cardoso, R.F. Dias, A.A. Freitas, E.M. Hungria, R.M. Oliveira, M. Collovati, S.G. Reed, M.S. Duthie, M.M. Stefani, Development of a quantitative rapid diagnostic test for multibacillary leprosy using smart phone technology., BMC Infect. Dis. 13 (2013) 497. doi:10.1186/1471-2334-13-497.

[12] Q. Wei, H. Qi, W. Luo, D. Tseng, S.J. Ki, Z. Wan, Z. Göröcs, L. a. Bentolila, T.T. Wu, R. Sun, A. Ozcan, Fluorescent imaging of single nanoparticles and viruses on a smart phone, ACS Nano. 7 (2013) 9147–9155. doi:10.1021/nn4037706.

[13] C.C. Stemple, S. V Angus, T.S. Park, J.-Y. Yoon, Smartphone-Based Optofluidic Lab-on-a-Chip for Detecting Pathogens from Blood., J. Lab. Autom. 19 (2013) 2211068213498241-. doi:10.1177/2211068213498241.

[14] A.F. Coskun, J. Wong, D. Khodadadi, R. Nagi, A. Tey, A. Ozcan, A personalized food allergen testing platform on a cellphone., Lab Chip. 13 (2013) 636–40. doi:10.1039/c2lc41152k.

[15] D.-S. Lee, B.G. Jeon, C. Ihm, J.-K. Park, M.Y. Jung, A simple and smart telemedicine device for developing regions: a pocket-sized colorimetric reader., Lab Chip. 11 (2011) 120–126. doi:10.1039/c0lc00209g.

[16] A.F. Coskun, R. Nagi, K. Sadeghi, S. Phillips, A. Ozcan, Albumin testing in urine using a smart-phone., Lab Chip. 13 (2013) 4231–8. doi:10.1039/c3lc50785h.

[17] A.K. Yetisen, J.L. Martinez-Hurtado, A. Garcia-Melendrez, F. Da Cruz Vasconcellos, C.R. Lowe, A smartphone algorithm with inter-phone repeatability for the analysis of colorimetric tests, Sensors Actuators, B Chem. 196 (2014) 156–160. doi:10.1016/j.snb.2014.01.077.

[18] J. Il Hong, B.-Y. Chang, Development of the smartphone-based colorimetry for multi-analyte sensing arrays., Lab Chip. 14 (2014) 1725–32. doi:10.1039/c3lc51451j.

[19] V. Oncescu, D. O’Dell, D. Erickson, Smartphone based health accessory for colorimetric detection of biomarkers in sweat and saliva., Lab Chip. 13 (2013) 3232–8.

doi:10.1039/c3lc50431j.

[20] D.P. Igoe, A. V. Parisi, B. Carter, Evaluating UVA aerosol optical depth using a smartphone camera, Photochem. Photobiol. 89 (2013) 1244–1248. doi:10.1111/php.12082.

[21] D.P. Igoe, A. Parisi, B. Carter, Smartphone-Based Android app for Determining UVA Aerosol Optical Depth and Direct Solar Irradiances, Photochem. Photobiol. 90 (2014) 233–237. doi:10.1111/php.12185.

[22] P.L. Urban, Universal electronics for miniature and automated chemical assays, Analyst. 140 (2015) 963–975. doi:10.1039/C4AN02013H.

[23] K. Pyrzynska, A. Pękal, Application of free radical diphenylpicrylhydrazyl (DPPH) to estimate the antioxidant capacity of food samples, Anal. Methods. 5 (2013) 4288–4295. doi:10.1039/c3ay40367j.

Figure 3

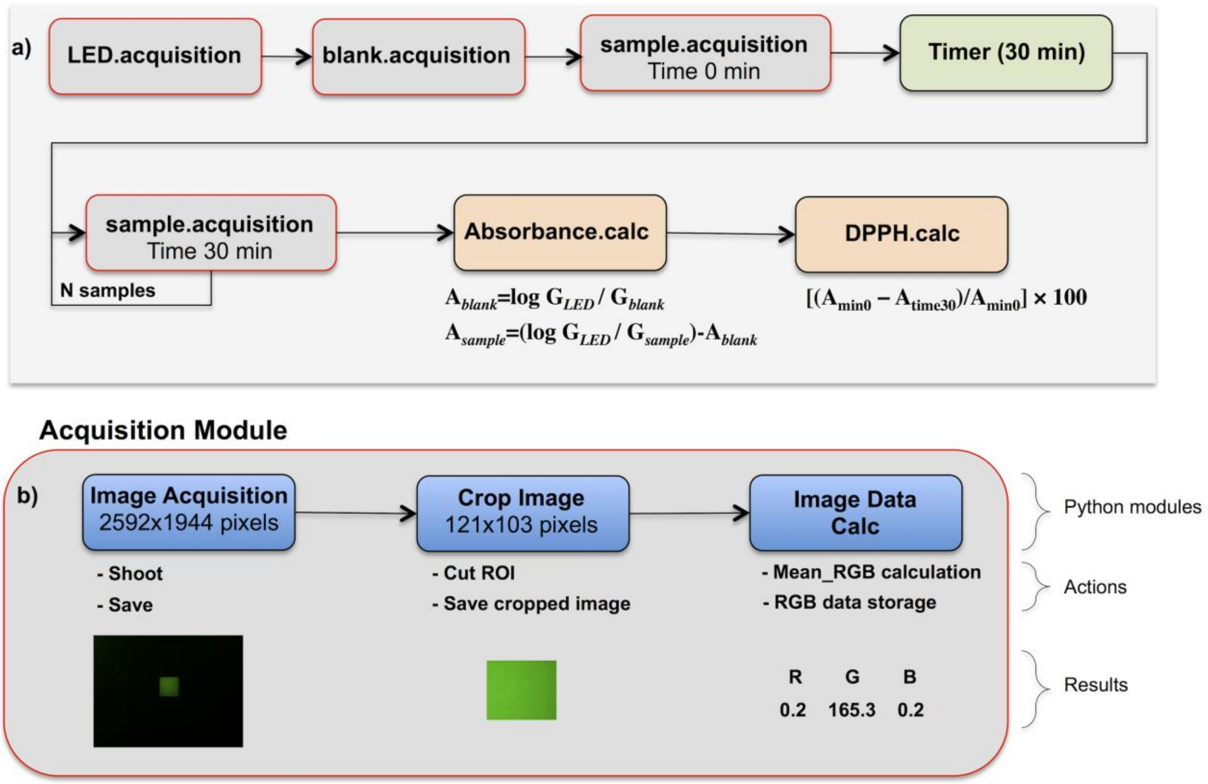

Fig 3. (A) Logical scheme of the python software managing the DPPH assay. Each block is called sequentially from the GUI (B). Detailed description of the acquisition module: each image was acquired with full-frame resolution and underwent a cropping process employed to derive the mean RGB values. ROI selection was automatized using a script based on the Python Image Library (PIL).

Figure 4

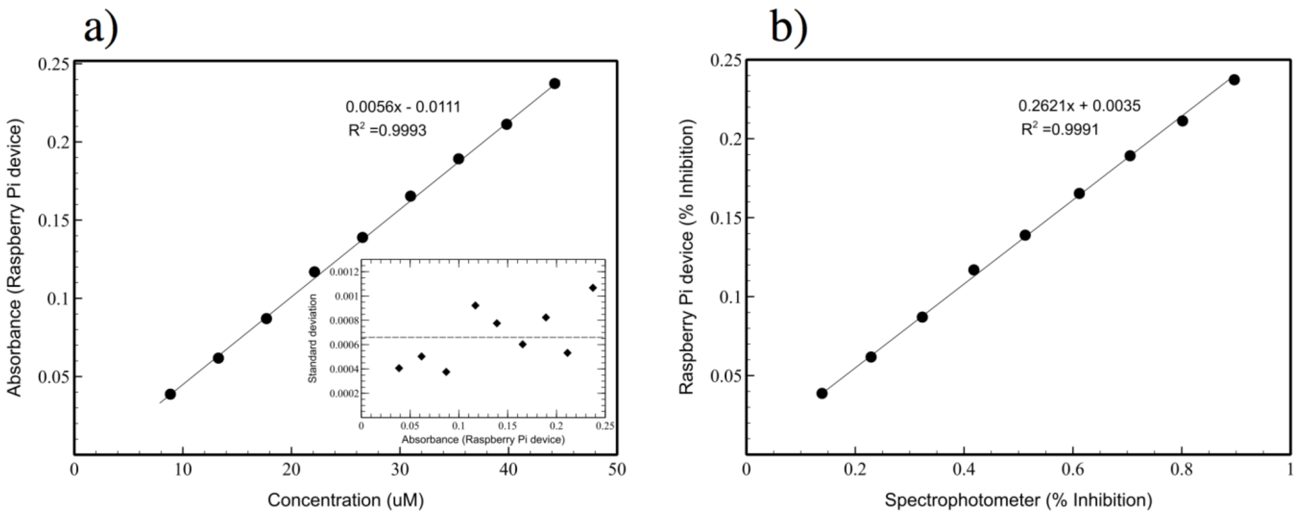

Fig. 4. (a) Calibration curve of amaranth dye using the Raspberry Pi-based device. The samples were prepared in order to have a concentration between 0.1 and 1.0. In order to evaluate the repeatability of the instrument, the measurements were performed 5 times and the standard deviation is reported in the boxed graph. (b) Correlation between the Raspberry Pi-based relative absorbance and the absorbance values obtained from the spectrophotometer.