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Colorimetric hand-held sensors and biosensors with a small digital camera as signal recorder, a review

Abstract: Sensors, biosensors, lateral flow immunoassays, portable thin-layer chromatography and similar devices for hand-held assay are tools suitable for field or out of laboratories assays of various analytes. The assays frequently exert a limit of detection and sensitivity close to more expensive and elaborative analytical methods. In recent years, huge progress has been made in the field of optical instruments where digital cameras or light sensitive chips serve for the measurement of color density. General availability of cameras, a decrease of prices and their integration into wide spectrum phones, tablets and computers give the promise of easy application of analytical methods where such cameras will be employed. This review summarizes research on hand-held assays where small cameras like the ones integrated into smartphones are used. Discussion about such assays, their practical applicability and relevant specifications are also written here.

1 Introduction

Currently, gas chromatography, liquid chromatography and high performance liquid chromatography are standard separation analytical methods that can be further combined with simple physical detectors (voltammetric, fluorimetric) or mass spectrometry and these methods have applicability for the measurement of a wide number of analytes, from simple inorganic or organic compounds to sized macromolecules of biological origin [1-10]. These methods represent direct competition or etalon for any newly developed assay. In the field of genetic material identification, polymerase chain reaction is the method of first choice, it can be performed for the identification of microorganisms, diagnosis of diseases, forensic search of perpetrators or victims, surveillance on genetically modified organisms, food control and others [11-18]. A lot of analytes can be determined by immunoassays. There are conditions where an antibody specific to the analyte should be available when the analyte is measured or there should be a specific antigen when antibodies are examined as a marker. Standard enzyme-linked immunosorbent assay (ELISA) and radioimmunoassay are examplesof the most common ones [19-25]. The aforementioned analytical methods are routinely available in hospitals, food and hygienic control, industrial and similar laboratories. Though there are of course other methods and devices, the mentioned standard analytical methods are seen as the main tool in the current analytical praxis.

This review is focused on the recent progress on hand-held assays like sensors, biosensors, lateral flow immunoassays, portable thin-layer chromatography and similar devices using small digital camera for output signal measurement. Tiny digital cameras like the one integrated in smartphones or portable touristic camera devices were considered here because they are cheap and suitable for everyday carrying. Analytical protocols can be of course based on highly sensitive photographic devices but these are out of scope of this paper. The hand-held assays are not direct competitors to the aforementioned standard analytical methods but they can support their findings, verify them, and they can also work in field and harsh conditions and represent a cheap and easy to perform type of assay. This review summarizes the recent findings on the issue

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of hand-held assays with digital camera as the output. Discussion about the practical role of hand-held assays, their differences and advantages, and their respective disadvantages to the standard methods are written here as well. The expected direction of the next research in this field is extrapolated from the knowledge of the current literature.

2 Color density and its measurement

Measurement of fluorescence and spectral absorbance are the most common methods and both of them are typically performed in cuvettes, microplates, flow through cells and so on. Compared to the standard methods, the assays presented in this review use standard digital camera for the outputting signal measuring. A standard digital camera is a device for making photographs and it is composed from common parts including optics (camera lens), the lens aperture, image sensor, shutter and image sensor. There are other optional parts like the control screen, viewfinder and camera flash for the support of taking pictures in less illuminated spaces. Just the flash is an important optional part when the camera is intended as part of a hand-held assay because the source of light is necessary to make the results repeatable and reproducible [26].

The most common digital output from a camera is jpeg (respectively its variants like jpg, jpe, jif, etc.) format. The jpeg contains 8 bit color information though some specific devices are able to produce jpegs with a higher value of information. The color information is kept in RGB color model which means that every pixel of the photograph contains information about Red (R), Green (G) and Blue (B) color channels [27]. The final color is achieved by the mixing of the three mentioned color channels. The 8 bit point to color depth is a discontinuous variable. The color depth is calculated as 2^n where n letter is an information about number of bits. For an 8 bit photograph, it has value 2^8 which is equal to 256 [28, 29]. It means that the color depth can have one of the 256 values equal to a number from 0 to 255. Every pixel of a photograph contains three numbers, one for the R channel, one for G and one for B. Compared to the standard 8 bit photograph, a photograph with only two bits contains four number for each channel, while a 12 bit photograph contains overall 4,096 color shades and 14 bit 16,384 color shades. 8 bit photography is typical for small and cheaper cameras with jpeg as its output. More expensive cameras can provide non-compressed raw data (typically 16 bit for digital single-lens reflex cameras) or non-compressed pictures in formats like tiff. Tiff format has ability to keep a high value of color depth. The so-called true color pictures and movies have color depth 24 bit corresponding with 16,777,216 values. Low value of color depth can represent a problem when a sample containing an analyte in concentration slightly above limit of detection should be distinguished from controls. Currently, 8 bit jpeg format is the most typical so any hand-held analytical method established on the standard camera devices integrated into phones, tablets or computers should consider this limitation when economic competitivity is a followed parameter. The general principle of digital photography and the shortcomings in its methodology were extensively reviewed in work by Pohanka, 2017 [26]. The idea of digital image processing was also extensively discussed in the cited papers [30-33]. Cameras can suffer from various problems with image quality. Apart from mistakes in assembly or construction shortcomings, there are also problems determined by the physical principle of photography: optical aberrations caused by lenses or noise and other negative impacts caused by detector chips. Cheaper cameras like the integrated one in other smart devices can suppress the aberrations and noise by software resulting in a partial correction but also in data loss. Collecting photographs in a raw format and not in standard picture format like jpeg is one way on how to avoid this effect. On the other hand, cheaper cameras do not support the collection of raw digital data from camera chip. When looking at specific shortcomings, the following can be considered: lenses can suffer from distortion (pincushion or barrel distortion), resulting in the whole image deformation. Edge parts of a picture can suffer from various chromatic aberrations appearing like colored lines. The edge parts of a picture can also darken due to the vignetting effect. The aforementioned effects can be suppressed by the option of a higher aperture number when the light conditions allow this. A negative impact on picture quality can also be due to conditions on the chip caused by light intensity and coloration in the moment of picture taking. Insufficient intensity of light leads to the setting of a higher ISO value but a higher ISO causes significant noise in the picture or camera record. There can also be problems with white balance resulting in incorrect color temperature on the recorded picture or camera record. A reliable source of light such as an integrated light diode or flash is necessary for avoiding problems with ISO and white balance. The white balance can also be switched off on most of devices which is desired in an analysis. The principle of information kept in a photograph is also explained in figure 1.
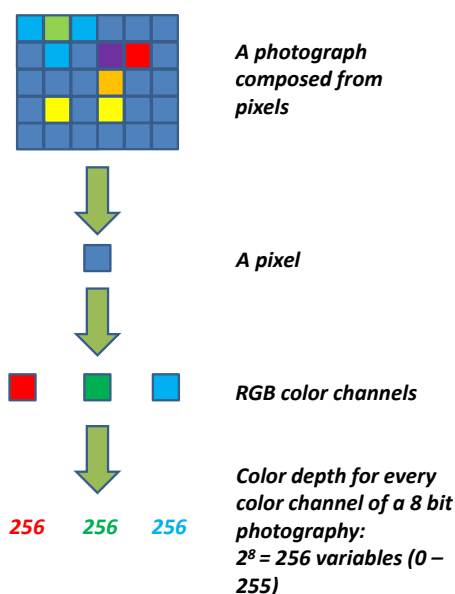
Figure 1 Explanation of digital information kept in an 8 bit photograph.

Digital pictures are a source of data for further analysis by many methods. The hand-held ones are discussed in the next chapters, but there are of course other ways to employ digital photography. Image processing has broad applicability in analysis of specific compound, chemical, physical and other processes or diagnosis of diseases. Such an application is of course set to specific conditions of the assay purpose. Characterization of skin structures [34, 35], glaucoma diagnosis [36, 37], study of cells in neuronal cultures [38], control of diamond's optical properties [39], pathological examinations [40], characterization of diffusing fluorescent molecules [41], classification of wheat grains and detection of poste damaged grain [42, 43], quantum steganography [44], improved microscopy and other typically biologist techniques [45-49], automated and robot self-controlled processed within Industry 4.0 strategy [50], fruit and vegetable quality control [51], biochemical analysis [52] and temperature distribution [53] are all methods linked to image processing. The digital camera can of course be linked to a bioassay such as in the following text: The bioassay can be based on various principles where an enzymatic or another chemical reaction provides a colored product or affinity interaction causing the catching of a fluorescent or colored trace. The general principle of a bioassay with digital camera as the output is depicted in figure 2. This review is focused on colorimetry and just the colorimetry is taken for an optimal method in connection of a small camera. The cameras can also be integrated into fluorimetry but there are higher demands on technical equipment, optical filters and light sources. There are known applications of fluorimetry using a digital camera and these techniques can be performed for example for oil droplets analysis [54], fluorimetry of eye applicable for diagnostics in ophthalmology [55], and DNA hybridization measurement [56, 57].
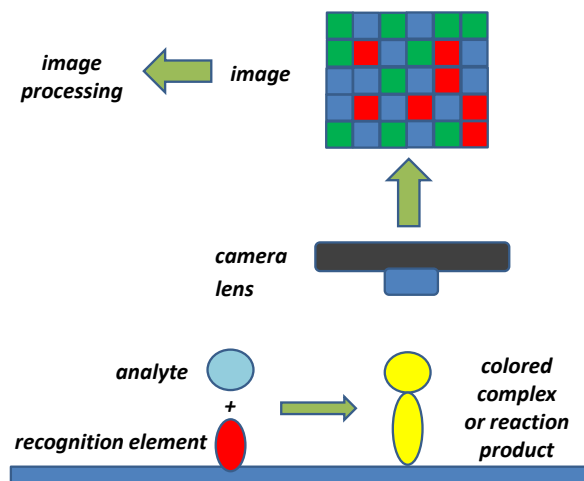

Figure 2 General principle of a bioassay with digital camera as the output.

3 Colorimetric biosensors with thin surfaces or small volumes and Thin-Layer Chromatography

A thin layer where coloration is formed due to an increase in the concentration of chromogenic analyte is common in thin layer chromatography. Thin layer chromatography can be easily linked with digital photography and illumination by UV light and this is optimal for this purpose. For instance, Tie-xin and Hong performed thin layer chromatography for cichoric acid from a plant *Echinacea purpurea* [58]. The authors used UV light and images recorded by a digital camera. The assay exerted a limit of detection of 0.067 µg and the calibration plot had quite good linearity and a coefficient of determination equal to 0.9917. In the mentioned study, a standard digital camera with a resolution of 5 megapixels and 10× optical zoom (no further specifications were provided in the paper) was used. Thin layer chromatography with a digital camera as the output was also tested by Simon and coworkers for an investigation of natural extracts [59]. The researchers extracted yellow bedstraw *Galium verum* into alcohol and made the principal component analysis, including the performance of thin layer chromatography, and recorded photographs under UV light with a wavelength of 254 and 366 nm. A single-lens reflex camera Nikon D3100 (Nikon, Tokyo, Japan)

with a standard Advanced photo System type-C (APS-C) sized Complementary Metal-Oxide Semiconductor (CMOS) chip was the digital data recording device. As a result, the authors described a specific chromatograph serving as fingerprints for the extracts. Thin layer chromatography with a digital camera as the output was found to be a reliable tool for various analytes and assays of amino acids after ninhydrin modification [60], creatinine labelled by iodine [61], leucine and isoleucine [62], amphetamine-like amines [63], and polycyclic aromatic hydrocarbons [64].

Thin layer can serve as a platform for enzymatic reactions, therefore it can be simply combined with color depth measurement by a digital camera. Such a concept was adapted for assays of butyrylcholinesterase as a biochemical marker in blood plasma and serum where it can help to reveal some types of poisonings or liver malfunctions [65]. The cited assay was based on color changes where indoxylacetate was converted by the enzyme butyrylcholinesterase to blue colored indigo and the whole assay was performed on small cuts of filter paper. Color depth was recognized from photographs taken by a camera integrated into a smartphone Samsung Galaxy S5 (Samsung, Seoul, Korea). The best sensitivity exerted the assay in R channel for which the limit of detection was equal to 3.09×10^{-6} kat/ml while B channel had a limit of detection of 4.67×10^{-6} kat/ml and G channel 4.36×10^{-6} kat/ml. The analytical parameters received in this study were quite close to standard spectrophotometry. An unpublished part of the experiment from the cited paper containing calibration of diluted serum is depicted as figure 3.
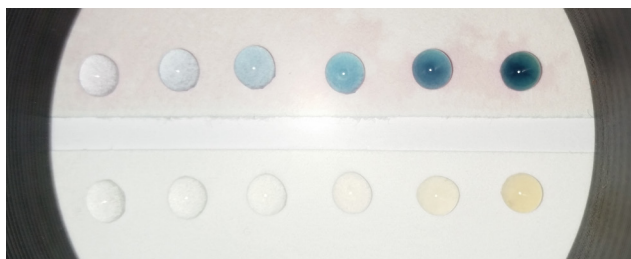

Figure 3 Measurement of plasma samples containing active butyrylcholinesterase. The photograph is an unpublished part of cited experiment [65] . The upper line contains saline solution diluted plasma samples and paper containing indoxylacetate, the lower part contains the same samples on a clean cut of paper. The photograph was taken by a smartphone camera and served for color depth determination when the photograph underwent image processing.

A low space of bubble wrap was selected in a study to establish a colorimetric method for measurement of glucose level [66]. The bubbles were filled with sol-gel, glucose oxidase, peroxidase and o-phenylenediamine dichloride as a chromogenic substrate. When added to a sample containing glucose, a cascade of reactions were initiated by the two immobilized enzymes and orange-brown end product from o-phenylenediamine was formed and photographed by a camera integrated into a smartphone Sony Xperia MT27i (Sony, Tokyo, Japan). The assay exerted a limit of detection of 0.75 mmol/l which is under the physiological level of glycemia (in health people 3.9 – 11.1 mmol/l) therefore the functionality of the method was reached. The assay fully correlated with standard spectrophotometry though the sensitivity was a little lower. A colorimetric assay working with small volume of analyte developed Marinho and coworkers for the determination of ethanol content in beverages [67]. They chose a smartphone camera as an analytical device and microtubes as a measuring cuvette. The coloration was formed by the addition of phenolphthalein in an alkaline medium into an alcohol beverage sample. The assay had a limit of detection 2.1 % (v/v) ethanol which is quite a high number, on the other hand it is enough to measure alcoholic beverages since the content of ethanol in them is significantly higher [68, 69]. A smartphone digital camera (Samsung Galaxy S5 by Samsung) served as a measuring device for the detection of neurotoxic carbofuran and consisted of cuts of filter paper with immobilized homogenate from psychrophilic bacteria [70]. The assay was based on a lipase mediated conversion of indoxylacetate to blue indigo, which was stopped by an irreversible enzyme inhibition in the presence of carbofuran. The limit of detection was equal to 3.72×10^{-8} mol/l for a 5 µl sized sample. A survey of mentioned methods is given in table 1.

4 Thin layer stational immunoassays and Lateral Flow Immunoassays

The recognition ability of antibodies is a principle of the immune response to an invading pathogen. The fact that antibodies are highly selective proteins can be used for various assays where an antigen is detected. The assay of prostate-specific antigen [71], infectious diseases [72-74], antibiotics [75], and food allergens [76] are relevant examples. The assay can also work in an opposing manner when the bioassay contains an antigen as a reagent or immobilized part and antibody is measured as an analyte. Immunosensors for celiac disease based on the detection of anti-transglutaminase antibodies [77] and detection of antibodies against infectious pathogens like *Francisella tularensis* [78] can be examined.

Immunosensors with a digital output work on a similar principle like the other immunoassays except for the signal measuring technique. The relevance of immunosensors

Table 1 Thin layer methods with digital camera on the output

Assay method	Used camera	Analyte	Limit of detection respective further specifications	References
thin layer chromatography	an unspecified camera with 5 megapixels and 10x optical zoom	cichoric acid from a plant *Echinacea purpurea*	0.067 µg	[58]
thin layer chromatography	standard single-lens reflex camera Nikon D3100 (Nikon, Tokyo, Japan) with CMOS chip of APS-C size	alcohol extract of *Galium verum*	fingerprints of individual extracts were described	[59]
colorimetric assay of butyrylcholinesterase activity on small cut of filter paper, indoxylacetate served as a chromogenic substrate	smartphone Samsung Galaxy S5 camera (Samsung, Seoul, South Korea)	butyrylcholinesterase	$3.09×10^{-6}$ kat/ml when R channel used for the assay	[65]
assay performed in bubble wraps containing glucose oxidase and peroxidase, color end products from o-phenylenediamine were formed	camera integrated into smartphone Sony Xperia MT27i (Sony, Tokyo, Japan)	glucose (glycemia)	0.75 mmol/l	[66]
assay performed in microtubes, phenolphthalein in alkaline medium was added to samples	an unspecified smartphone camera	ethanol in alcoholic beverages	2.1 % (v/v)	[67]
colorimetric assay of lipase activity on small cut of filter paper, indoxylacetate served as a chromogenic substrate, the reaction was stopped in the presence of carbofuran	smartphone Samsung Galaxy S5 camera (Samsung, Seoul, South Korea)	carbofuran	37.2 nmol/ for a sample 5 µl	[70]

with a digital output can be learned from the following examples. Streptomycin was, for instance, detected by colorimetry with a smartphone as the output [79]. The assay worked on the principle of interaction between an aptamer and streptomycin, the excess aptamer hybridized with complementary DNA and the resulting double stranded DNA interacted with green fluorescence providing the color SYBR Green I. The assay exerted a linear range of 0.1 – 100 µmol/l and a limit of detection of 94 nmol/l. An immunosensor with digital output was constructed for the measurement of alkaline phosphatase which was intended to serve in milk quality control [80]. The assay was based on alkaline phosphatase separation from a sample on paper modified with anti-alkaline phosphatase antibodies. After separation, alkaline phosphatase converts chromogenic substrate 5-bromo-4-chloro 3-indolyl phosphate, providing blue-green coloration which was observable via a smartphone camera. The assay had a two-order sized linear response from 10 to1,000 U/ml and exerted a limit of detection of 0.87 U/ml. Another assay on alkaline phosphatase was performed by Yu and coworkers [81]. In this assay, the paper strip contained gold

nanoparticles modified with phosphotyrosine and a testing line with antibodies specific to phosphotyrosine. Alkaline phosphatase from a sample causes the dephosphorylation of nanoparticles and affects the interaction between nanoparticles and immobilized antibodies. The colored spots were recorded and analyzed by a smartphone. The assay exerted a dynamic range of 0.1 – 150 U/l and a limit of detection of 0.1 U/l. Immunoassays have good specifications to reveal sized analytes because the preparation of antibodies against sized antigens is easier and the sized antigens can be precipitated by antibodies because more immunoglobulins can bind to one sized structure like cells and large proteins. The precipitation is conditioned by fact that immunoglobulins have more paratopes binding antigen. It can be from two (e.g. immunoglobulin G – one subunit with two paratopes) up to ten (e.g. immunoglobulin M – five subunit, each two paratopes). The detection of the bacterium *Escherichia coli* was described in the paper by Sai and coworkers [82]. The assay worked on the principle of a paper with an antibody capturing *E. coli* from samples and the captured bacterium was then labelled with gold nanoparticles modified with another anti *E. coli* antibody.

Formed spots can be further highlighted by the addition of silver ions which are reduced by gold nanoparticles to metallic silver. The assay was performed on standard filter papers and a cell phone camera (no further specification provided in the paper) served for image processing. The limit of detection for the assay was equal to 57 CFU/ml. This result is quite close to the standard ELISA method for bacteria detection [83], therefore the digital camera assay could substitute the ELISA.
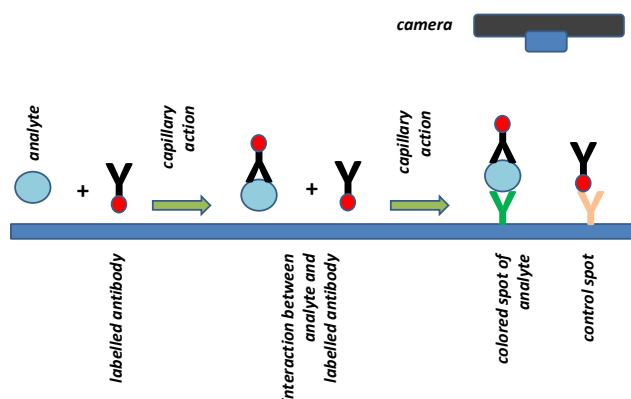

Figure 4 General principle of lateral flow immunoassay with digital camera on the output.

Colorimetric immunoassays with signal scoring by a naked eye can be easily improved by the addition of a digital camera as the output. Lateral flow immunoassays also known as lateral flow tests are designed as standard hand-held methods and the formed spots can be characterized by the digital camera. In principle, the lateral flow immunoassay consists of sample migration by capillary action up to the spot where it is captured by an immobilized antibody, another labelled (fluorescent nanoparticle, fluorescent dye etc) antibody finalizes the formed sandwich and it causes that the spot is visible due to coloration [20, 84-88]. The control zone is represented by an antibody against the labelled antibody. The control zone should be formed in any case regardless of the presence of analyte in the sample. As an alternative, the assay can also be performed on the principle of single stranded sequences of DNA hybridization and such a method can also be combined with a digital camera [57, 89-91]. Single nucleotide polymorphism can be scrutinized by the digital camera based lateral flow immunoassay [92-96]. Such devices are an alternative to a standard lateral flow immunoassay.
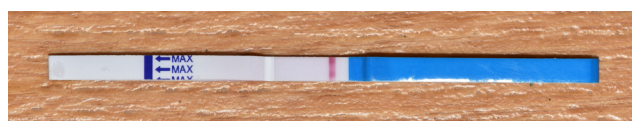

Figure 5 Example of a commercially available lateral flow immunoassay strip - human chorionic gonadotropin test.

The efficacy of an assay where a digital camera is combined with a lateral flow immunoassay is expected to be higher than the lateral flow immunoassay alone [97, 98]. A general principle of a lateral flow immunoassay in combination with a digital camera is depicted as figure 4. An example of a commercially available lateral flow immunoassay test strip (human chorionic gonadotropin test) is in figure 5. The relevance of the combination can be learned from following examples. Lateral flow immunoassay was for instance chosen as a method for the detection of cocaine [99]. In the cited paper, a magnetic lateral flow immunoassay was performed on urine samples for the presence of cocaine. The assay worked on the principle of competition between cocaine and conjugate cocaine – bovine albumin. Magnetic beads covered with anti-cocaine antibodies reacted with cocaine or complex cocaine – the bovine albumin formed a gray line on the support pad. Standard smartphone camera served for signal scaling. The assay had limit of detection 3 nmol/l and linear range 5 – 500 ng/ml. A lateral flow immunoassay with a digital camera as the output was introduced by Xia and coworkers for chloramphenicol residue assay [100]. The assay exerted limit of detection 0.03 ng/ml. A smart phone Samsung Galaxy S7 Edge with a digital camera and lateral flow immunoassay together were performed for albumin assay [101]. The assay had a limit of detection of 1.71 pg/ml and limit of quantification 26.48 pg/ml. In another experiment, digoxigenin was assayed by lateral flow chromatography [102]. Digoxigenin is a toxic plant steroid forming conjugates to sugars and known for immunogenicity. The assay was mediated by CMOS camera from smartphone iPhone 5S (Apple, Cupertino, California, USA) and used an antibody - gold nanoparticles. The best limit of detection was 14.4 nmol/l and the limit of quantification 19.1 nmol/l. The assay by smartphone was compared with a professional digital colorimeter BioImager (ChemStudio Plus, Analytic Jena, Jena, Germany). When the two assays compared, the professional colorimeter proved to have better specifications (sensitivity, limits of detection and quantification) but the advantages were not significant and considering price and simplicity, make the smartphone camera a viable competitor to the standard colorimetry. A lateral flow immunoassay improved by signal measuring by a smartphone camera was also performed for the determination of 8-Hydroxy-2'-Deoxyguanosine [103] and the identification of antibiotic resistance with the combination of a lateral flow assay, polymerase chain reaction and a smartphone [104]. An overview of the aforementioned immunoassays is given in table 2.

Table 2 Immunoassays with digital camera on the output

Assay method	Used camera	Analyte	Limit of detection respective further specifications	References
an aptamer interacted with streptomycin, the excess aptamer reacted with complementary DNA and then SYBR Green I providing green fluorescence	an unspecified smartphone camera	streptomycin	linear range 0.1 – 100 µmol/l and limit of detection 94 nmol/l	[79]
alkaline phosphatase was separated on papers modified with specific antibodies; in the second step, 5-bromo-4-chloro 3-indolyl phosphate provided blue-green coloration due to the enzyme activity	an unspecified smartphone camera	alkaline phosphatase	linear response from 10 to 1,000 U/ml, limit of detection 0.87 U/ml	[80]
strip containing gold nanoparticles modified with phosphotyrosine and testing line with antibodies specific to phosphotyrosine; alkaline phosphatase from a sample caused dephosphorylation and affecte the interaction between nanoparticles and antibodies	an unspecified smartphone camera	alkaline phosphatase	dynamic range 0.1 – 150 U/l and limit of detection 0.1 U/l	[81]
paper with antibodies against *Escherichia coli* captured the bacterium, gold nanoparticles with antibodies labelled captured bacterium, reduction of silver ions make the spots more visible	an unspecified cell phone camera	*E. coli*	limit of detection 57 CFU/ml	[82]
lateral flow immunoassay	an unspecified smartphone camera	cocaine	limit of detection 3 nmol/l, linear range 5 – 500 ng/ml	[99]
lateral flow immunoassay	an unspecified digital camera	chloramphenicol	limit of detection 0.03 ng/ml	[100]
lateral flow immunoassay	smartphone camera Samsung Galaxy S7 Edge (Samsung, Seoul, South Korea)	albumin	limit of detection 1.71 pg/ml, limit of quantification 26.48 pg/ml	[101]
lateral flow immunoassay	smartphone camera iPhone 5S (Apple, Cupertino, Ca, USA)	digoxigenin	limit of detection 14.4 nmol/l, limit of quantification 19.1 nmol/l	[102]

5 Conclusions

Digital camera based analytical devices appear to be promising for current praxis and would meet demands from various laboratories including clinical, control of manufacturing processes, assay of pollutants, hygienic control etc. It is not expected that camera based hand-held assays will replace standard instrumental methods like chromatography and mass spectrometry but they represent a support to the standard devices and can replace them under some specific conditions like field assay or assays performed under emergency conditions. The major advantage of the hand-held assays with digital cameras is the overall simplicity, low price and general availability. An increasing role of hand-held assays with digital cameras is expected in the next few years. Further development of analytical methods based on digital camera can also be accelerated by other techniques like 3D printing [105-107]. The combination of smartphone camera and individually manufactured parts by 3D printing technique would make analytical techniques available for nearly everyone.

Acknowledgements

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