DIGITAL IMAGE ANALYSIS FOR DETECHIP® CODE DETERMINATION

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ABSTRACT

DETECHIP® is a molecular sensing array used for identification of a large variety of substances. Previous methodology for the analysis of DETECHIP® used human vision to distinguish color changes induced by the presence of the analyte of interest. This paper describes several analysis techniques using digital images of DETECHIP®. Both a digital camera and flatbed desktop photo scanner were used to obtain Jpeg images. Color information within these digital images was obtained through the measurement of red-green-blue (RGB) values using software such as GIMP, Photoshop and ImageJ. Several different techniques were used to evaluate these color changes. It was determined that the flatbed scanner produced in the clearest and more reproducible images. Furthermore, codes obtained using a macro written for use within ImageJ showed improved consistency versus pervious methods.

KEYWORDS

DETECHIP®, Molecular Sensing Array, Narcotics Detection, Image Analysis, RGB Color Signal

1. INTRODUCTION

Designed for lab and field use, DETECHIP® is a mix-and-measure assay that is capable of providing both colorimetric and fluorescent signals for the rapid detection and identification of molecules of emerging interest such as narcotics, narcotics with cutting agents, over the counter medications, volatile organic compounds, explosives and the intermediates used to make them, microbial metabolites, and environmental contaminants like pesticides [1, 2]. The term DETECHIP® (short for detection chip) combines the concept of small molecule detection with the use of an array of chemical indicators. There are many applications that require a quick, sensitive and selective detection system for specific compounds, including alerting security officers to the presence of explosives and their precursors, screening for weapons of mass destruction, testing biological fluids for illegal compounds, and detection and quantification of sports doping compounds. Colorimetric assays (i.e. "spot tests") offer speed, simplicity of operation, portability, and affordability [3-6]. The stability and versatility of these spot tests enable lab scientists or field personnel to "triage" samples and select those for additional analysis, but they do not provide positive identification.

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GC-MS [7-9] is the most widely used method to detect these types of substances, but sample introduction, miniaturization, and the need for skilled operators remain a challenge. Furthermore, high-resolution instruments and expensive additional assays such as isotope ratio mass spectrometry (GC-IRMS) are often required to distinguish between similar compounds. Highly specific tests, such as enzyme-linked immunosorbent assays (ELISA) typically involve chromophore reporters that produce a color, fluorescent, or electro-chemiluminescent change to indicate the presence of specific antigen [10]. While these immunoassays offer extremely high sensitivity, they are also expensive, non-quantitative, and have limited shelf life because they are protein-based and water or humidity sensitive.

None of the described methods are practical for screening thousands of compounds spanning several different molecular classes, and it is this need that DETECHIP® fills. DETECHIP® offers a simple, sensitive, selective, and affordable alternative to existing technologies for the detection of analytes including heroin, cocaine, tetrahydrocannabinoldate (THC) from marijuana, as well as date-rape and club drugs such as flunitrazepam, gamma-hydroxy butyric acid (GHB), or methamphetamine. Significantly, the same system also uniquely identified the explosive trinitrotoluene (TNT), five organic compounds produced by spoilage yeast in beer and wine, as well as over 25 pesticides that are an environmental concern to the U.K. government [11]. Shown to be contactless, portable, inexpensive, DETECHIP® can be adapted to identify a number different classes of substances. Unlike other color tests, which result in a single 'yes' or 'no' response intended to signify that a functional group is present, e.g. the amino group of a narcotic [3, 12], DETECHIP® provides many simultaneous responses, allowing users to quickly characterize and identify suspect materials by assembling a unique, substance specific, binary code composed of '1' and '0'. In this code, '1' represents a change in color or fluorescence, while '0' represents no change. **Figure 1** summarizes the assembly and interpretation of DETECHIP®. First, the DETECHIP® sensors, represented by the blue drops in **Figure 1a**, are placed into the wells of the 96-well plate with each row of twelve containing a unique DETECHIP® sensor. The sensors are then exposed to the analyte of interest, represented by the red drops in **Figure 1b**. The analyte is added to alternating 8-well columns to provide a control well for later comparison. **Figure 1c** represents a well that has experienced after analyte addition, and includes a detection method. The ability for the simultaneous detection of controls and suspect materials is unique to DETECHIP®. **Figure 1d** includes an image of DETECHIP® in its entirety illustrating color changes as well as fluorescent changes when exposed to UV light.

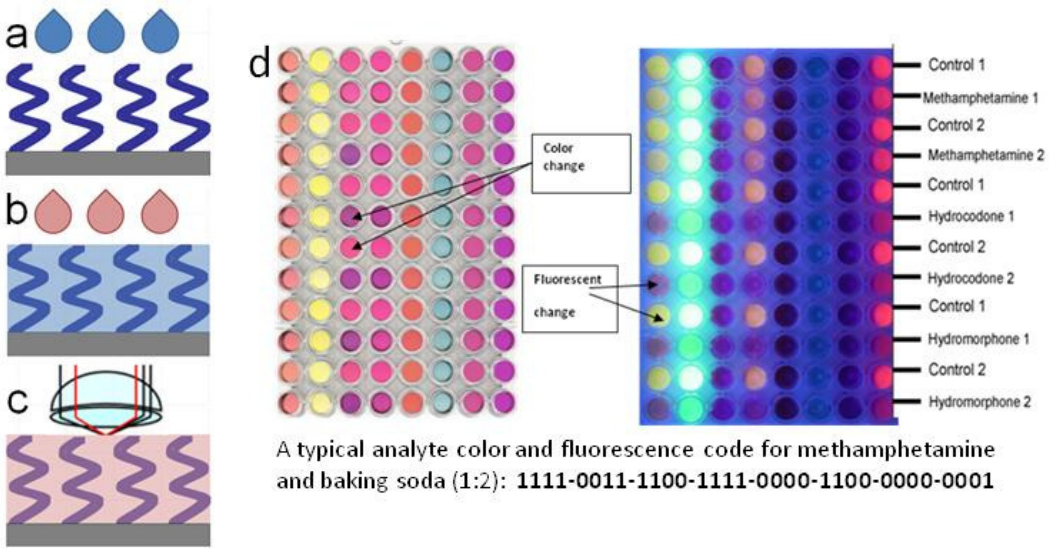

Figure 1. Illustration of sensing principles for parallel monitoring/readout of molecular interactions on

DETECHIP® using image analysis of color images: (a) placement of DETECHIP® elements (blue drops) into 96-well plates (b) exposure of DETECHIP® to analyte (red drops) (c) measurement of RGB change with image software. (d) A typical DETECHIP® ready for analysis. Color (CC) and fluorescence (FC) changes in the sample well relative to the control well are noted (arrows). These changes are recorded as a binary code. A "0" indicates no change while "1" denotes a change in the sample. A representative code for methamphetamine in the presence of an adulterant (baking soda) is **1111-0011-1111-0000-1100-0000-0001**.

Affording individual codes for the multitude of compounds listed in **Table 1**, DETECHIP® has the unique ability to detect and discriminate substances in many difference classes: over-the-counter (OTC) medications, explosives, pesticides, food spoilage metabolites, drugs laced with cutting agents, and various other organic molecules [1-4, 12].

Table 1. List of substances currently under investigation using DETECHIP®.

DRUG OR NARCOTIC	OVER THE COUNTER	DRUGS WITH CUTTING AGENTS	PESTICIDES
Phenylcyclohexyl piperidine	24 Hour allergy relief D	Cocaine/Baking soda (1:1)	2,4 - Diiodo-4-hydroxybenzonitrile
Caffeine	24 Hour allergy relief D	Cocaine/Dextrose (1:1)	2-Hydroxy-1-(2-Hydroxy-4-Sulpho-1-Napthlazo)-3-Napthoic Acid
Cocaine	Caffeine	Cocaine/Epsom salt (1:1)	3-(3,4-dichlorophenyl)-1,1-dimethylurea
Codeine	DG Antacid tablet	Cocaine/Glucose (1:1)	3-(4-chlorophenyl)-1-methoxy-1-methylurea
D-Amphetamine sulfate	DHEA (Dehydroepiandrosterone)	Cocaine/Lactose (1:1)	3,5 - Diiodo-4-hydroxybenzonitrile
Fentanyl	Enteric coated aspirin	Cocaine/Lidocane (1:1)	4,7 -Diphenyl-1,10-phenanthroline disulphonic acid disodium salt
Flunitrazepam	Equate allergy medication	Cocaine/Mannitol (1:1)	4-Chloro-o-tolyloxyacetic acid
Hydrocodone	Equate naproxen sodium	Cocaine/Methylsulfone (1:1)	4-Dimethylaminobenzylidene-rhodanine
Hydromorphone	Equate sleep aid	Cocaine/Phenacetin (1:1)	Asulam
Ketamine	Glucosamine Chondroitin	Cocaine/Powdered milk (1:1)	Atrazine
L-Alphacetylmethadol	Ibuprofen	Cocaine/Powdered sugar (1:1)	Bromoxynil
Methadone	Jet-alert	Cocaine/Starch (1:1)	Chlorotoluron
Methamphetamine	L-Glutamine	Cocaine/Sugar (1:1)	DDE
Methylphenidate	Multivitamin	Cocaine/Talc (1:1)	Dichlorprop
Morphine	Phenacetin	Meth/Baking soda (1:1)	Dithizone
Quinine	Suphedrine sinus headache	Meth/Dextrose (1:1)	Diuron
Thebaine	Tylenol cold day	Meth/Epsom salt (1:1)	Endosulfan
	Tylenol cold night	Meth/Glucose (1:1)	Endrin
		Meth/Lactose (1:1)	Gamma-BHC
		Meth/Lidocane (1:1)	Hexamethyldisilazane
		Meth/Mannitol (1:1)	Ioxynil
EXPLOSIVES	**WINE CORK METABOLITES**	Meth/Methylsulfone (1:1)	Isoproturon
TNT (Trinitrotoluene)	Guaiacol	Meth/Phenacetin (1:1)	Linuron
	Geosmin	Meth/Powdered milk (1:1)	MCPA
	TCA	Meth/Powdered sugar (1:1)	MCPB
	4-Ethylguaiacol	Meth/Starch (1:1)	Mecoprop
	4-Ethylphenol	Meth/Sugar (1:1)	p.p'-DDT
		Meth/Talc (1:1)	Simazine

Highly successful in its current form, DETECHIP®'s 32 digit binary code for each analyte is obtained by a person visually inspecting each analyte well and comparing it to the control well. Unfortunately in some tests, differences in human vision and subjective interpretation produce inconsistent codes for identical analytes. This variability can be minimized through plate analysis by multiple people with the consensus determining the binary code. Although providing highly consistent results[13], this method is labor, time, and personnel intensive. In order to circumvent this timely process and eliminate human variability, and thereby decreasing the occurrence of inconsistent codes and false positives, image analysis techniques were employed.

In the last several years, the use of colorimetric sensing, using red-green-blue (RGB) values, as a detection method for digital array images has increased in popularity [13-19]. Various analytes can be detected using RGB color space including pigments of green beans [19], nitrates [17], sugars [15], peroxide vapors [16], and biogenic amines [18]. "Optoelectronic noses" have been reported in conjunction with image analysis and have shown to be an effective detection tool for odorants and gases, but not for abused substances and other analytes of interest to us [20-23]. Used to identify the analyte and determine concentration, these digital image analysis methods are based on color differences as perceived by the software. Examples of software programs that have been used for this type of analysis include ImageJ [24], GIMP [25], and Adobe Photoshop.

Utilizing similar photo analysis techniques, the hypothesis for DETECHIP® is that digital measurements of RGB values are more objective than previous measurements made by visual interpretation and will therefore eliminate errors caused by differences in human vision and subjective interpretation of color. Although the original DETECHIP® format employed both color and fluorescent changes to produce a 32-digit binary code [1, 2, 12], using image analysis will eliminate the fluorescence aspect of DETECHIP®. The code will therefore rely solely on the interpretation of color changes to produce a now 16-digit binary code. This manuscript presents several different digital image analysis techniques for the interpretation of DETECHIP®, using commercially available or in-house designed image software for the measurement of changes in RGB intensities after exposure to the analyte of interest. The resulting codes and their associated accuracy and precision will be compared against codes previously established through visual interpretation. This approach will be advantageous in the progression towards automation of DETECHIP®.

2. MATERIALS

DETECHIP® plates were prepared for triplicate analysis of a single analyte at a final concentration of 25 mM, as opposed to previous plates that were prepared with three separate analytes. This allowed for the investigation of consistency in the determined codes. Digital images of DETECHIP® were obtained using either a Canon EOS Rebel T1 EOS 500D camera with an EF 50mm f/2.5 compact-macro lens, or an Epson V700 Photo Scanner. Analysis of the resulting digital images was done using ImageJ, and gimp software programs, as well as Adobe Photoshop, MATLAB, Microsoft Excel, and an in-house designed macro.

3. METHODS

3.1. Photo Analysis Method

Photos of the DETECHIP® plates were taken using a Canon EOS Rebel T1 EOS 500D camera with an EF 50mm f/2.5 compact-macro lens. Digital photo analysis was performed on a JPEG file that was created using a 10:1 compression algorithm with negligible loss in image quality. The first step of the analysis process included pre-processing of the image file to locate exact regions with in the wells, which contained control and analyte samples. The preprocessing steps

included cropping the image file, scaling down the resolution to 500 x 800 pixels, and determining the center of each sample well. A MATLAB program [26] was used to identify the centers of each well. The results, however, were not highly compelling. Therefore, an interpolation program was written in Perl to not only filter out the improperly identified centers but also to detect missing centers. The centers for each of the 96 wells were highly accurate, all being checked visually by plotting the centers determined by the algorithm of the image. A MATLAB program was written to determine the RGB intensities for each pixel in a circular region around these centers and thus the total RGB intensity for every well. The average RGB values were calculated based on four different plates of the same drug/sensor combination. If the error bars (standard deviation) of the RGB intensities for the analyte did not overlap with those for the controls, it was counted as a statistically significant change and given a '1' for a code, but if there was an overlap of the standard deviation between the analyte and either one or both of the controls, then a '0' was assigned. The codes were then compared between the original visual analysis method, and the new digital analysis protocol. The photo analysis method measures the RGB intensities but not fluorescence, thus in order to make a comparison between the color changes performed by visual inspection with the digital photo analysis, the codes were changed to account only for color changes, thereby reducing the original 32-digit code to the now 16 digit code.

3.2. Scanned Image Analysis

A DETECHIP® 96 well-plate containing three identical tests of one analyte of interest was scanned using an Epson V700 Photo Scanner. The positive film scanned image was 1350x1983 pixels and was saved as a TIFF image. This scanned image was then analyzed using three image analysis techniques, all of which employed the use RGB values. As previously stated, in reference to the photo analysis method, the omission of fluorescence reduced the resulting code from 32 to 16 digits.

3.3 Subtraction Method

For image analysis via the subtraction method, the scanned image, saved as a TIFF file, was opened in GIMP (GNU Image Manipulation Program), a free online imaging software program. As seen in **Figure 2**, two separate images were created for the original scanned TIFF file. In the first, the analyte wells were eliminated, leaving only the control wells, while in the second only the analyte wells remain. These two images were then subtracted using the image subtraction filter in the GIMP software, setting the analyte image as the master (**Figure 2a**) and the control image as the slave (**Figure 2b**). By subtracting out the color of the control well from that of the analyte well, this qualitative method reveals the color difference caused by the addition of analyte. Any color remaining in the well indicates a color change and results in a value of '1' in the final code. If no color change exists, the resulting image will be white, corresponding to a code value of '0'. Interference does exist due to the presence of the wells themselves in the image, but this can be eliminated through the use of image masking. The following method, referred to as the Masking Method, takes the qualitative nature of the image subtraction method and adapts it to produce quantitative method for code determination.

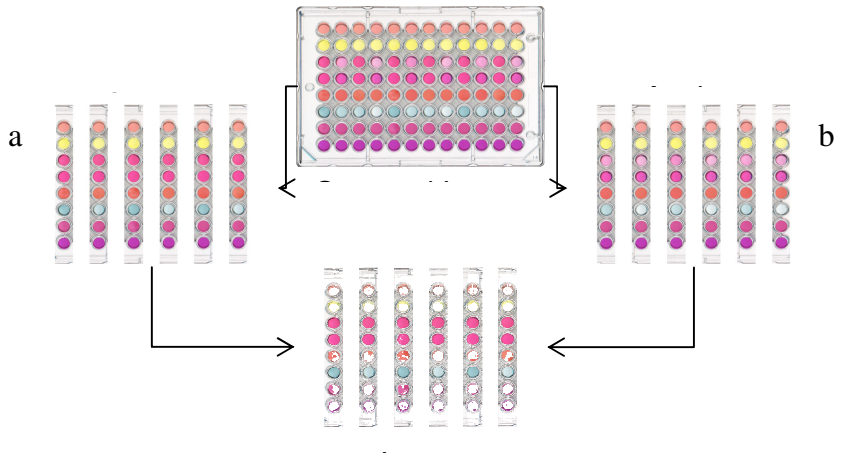

Figure 2. Example of an image subtraction assay. The scanned image of the control and analyte wells is subtracted from each other. The resulting image subtraction is then analyzed for wells that are colorless versus colored. A colorless well indicates that the control and analyte well are the same and codes for a '0'. A colored well indicates that there is a difference in color between the analyte and color wells and codes for a '1'.

3.4 Masking Method

As seen in **Figure 3**, the masking method begins with the positive film scanned image of a DETECHIP® 96 well-plate. The GIMP software was the used to create a black mask with the elimination of 96 perfect circles, each with a diameter of 68 pixels. The mask is layered overtop the image of the DETECHIP® 96 well-plate. The new masked image was then opened for further analysis in ImageJ. Using the threshold selection tool, all 96 circles were selected and analyzed for average RGB values, area, and standard deviation. Consistency across the three tests was analyzed through comparison of values ± 1 standard deviation. Overlap in these values indicated that the three tests gave statistically identical results. Additionally, a comparison of the control and analyte well for each dye was also done for each of the three tests, altering the standard deviation to facilitate consistency across these three tests. Initially, the three tests were analyzed for consistency at one standard deviation, compiling a code for each test. These three codes were then compared. If discrepancies appeared between the codes, the value of the standard deviation was decreased by a factor of 0.1, after which the values were reanalyzed for overlap and the codes were determined again. This process continues until all three tests resulted in identical codes.

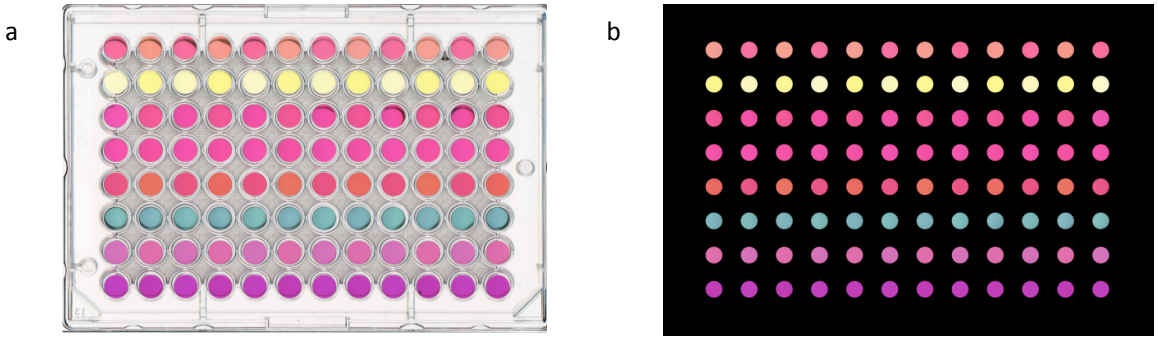

Figure 3. Scanned images of caffeine on a DETECHIP® plate: (a) original scanned image (b) image after proper orientation and masking.

3.5 Macro Method

After opening a scanned image of a DETECHIP® 96 well-plate in the ImageJ program, the image was properly oriented for analysis using a newly designed macro, created in-house, through modification of previously published work [17]. This macro was designed to select a circular area (47 x 50 pixels) in the center of each well. Within the selected region of the well, each pixel was analyzed to obtain a value for Red. These values were used to calculate an average Red value for the entire selected region of the well. This process was then repeated on the same region to obtain average values for Green and Blue. This sequence of measurements was preformed on all 96 wells. The analysis of both analyte and control wells allowed for simultaneous comparison of these color values. The macro was programmed to produce a '1' if there is a sizeable difference in any of the Red, Green, or Blue values between the control and the analyte wells. The term 'sizable' refers to a value larger than the set color threshold. This threshold value, optimized through experimentation, provides a quantifiable cutoff for what is considered a color change. Differences larger than this threshold are considered sizeable; producing a '1' in the code, while differences smaller than this value characterized as 'no change', producing a '0' in the code.

4. RESULTS

4.1 Photo Analysis Method

Due to the fact that the photo analysis method measures only the RGB color intensities but not fluorescence, the original 32-digit DETECHIP® codes were reduced to 16 digits. This allowed for a direct comparison between the color changes recorded by human visual inspection and the codes determined though digital photo analysis. The average RGB values were calculated based on four different plates of the same drug/molecular sensor combination. **Figure 4** shows results of the RGB values of analyte wells versus control wells. If the error bars (plus or minus one standard deviation) of the RGB intensities for the analyte did not overlap with those for the controls, was counted as a statistically significant change and given a '1' for a code. If there was a standard deviation overlap between the analyte and either one or both of the controls, then a '0' was assigned.

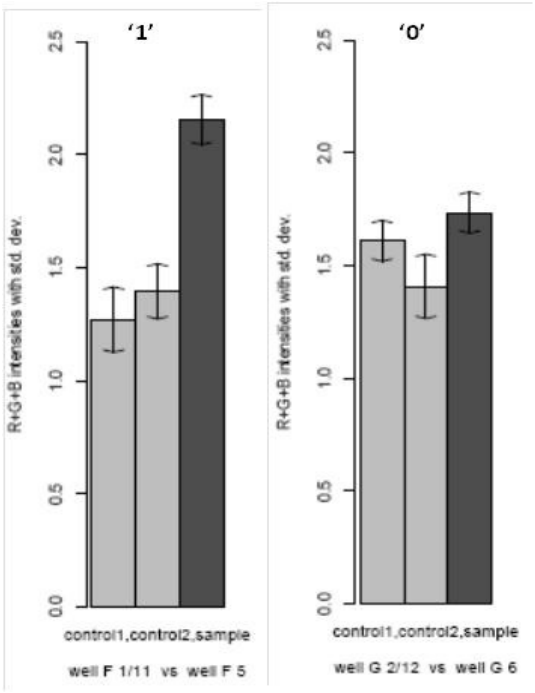

Figure 4. Example of code assembly using photo analysis method. The error bars on the left are not overlapping, thus the code is "1". The error bars on the right are overlapping and thus code a "0". The resulting codes are compared with codes previously determined using visual interpretation.

Overall, the results of visual inspection versus digital photo analysis matched well when codes produced by the two methods were compared. In some instances, as seen in **Table 2**, the digital photo analysis indicated a significant color change that was not visible by eye (i.e. methamphetamine at positions 4, 11, and 12 in **Table 2**).

Table 2. Analytes from six molecular classes that gave unique 32-digit codes using the 8-sensor Macro-DETECHIP®.

Methamphetamine	
Visual	0100111100000000
Digital	0101111100110000
Hydromorphone	
Visual	1100000000101000
Digital	1100111000110000
Methadone	
Visual	1100111111111101
Digital	1111111111111001
Hydrocodone	
Visual	1111111000110000
Digital	0010111000110000

Highlighted in grey are three cases in contrast, whereby color changes were seen visually, but were not detected by digital photo analysis. As an example, for hydrocodone in buffer B, sensor DC2 (fourth digit in hydrocodone code, **Table 2**) displays a significant difference in blue intensity between the analyte and the control. For hydromorphone and methadone, the color intensity values were very subtle, while color differences between analyte and control were more difficult to detect.

The photos used for photoanalysis suffered of parallax and shading of the wells which led to large variations and lack of consistency. In order to improve the image quality and consistency of the assay, the plates were scanned with a flatbed scanner in transparency mode, leading to less parallax, more clear and consistent images, less shading differences, and more consistent lighting. **Figure 5** demonstrates the improvement of image quality when DETECHIP® was scanned instead of photographed.

Figure 5. Left: DETECHIP® photographed with a Canon camera. Right: DETECHIP® scanned with a flatbed scanner.

4.2 Masking Method

This method was built upon the initial findings of the image subtraction method, after it was determined that a more quantitative method was required. The scanned image of the DETECHIP® 96 well-plate was analyzed in ImageJ not only to determine a code for the analyte of interest, but also to look into the consistency between the three tests of the analyte within the plate. For code determination, values for average RGB intensity and standard deviation of RGB intensity was determined for each well. This was accomplished through software analysis of all pixels within each well. The average RGB value and associated standard deviation for each analyte well was compared to the corresponding control using Microsoft Excel. Similar to the photo analysis method, if the error bars – representing ±1 standard deviation value – overlapped, a value of '0' was assigned to the code – signifying that there was no appreciable difference in color between the two wells. However, if the errors bars did not overlap, a value of '1' was assigned to the code, thus designating a color change.

The code for the analyte of interest was determined at several multiples of the standard deviation value, beginning at ±1 standard deviation and reducing this value by a factor of 0.1 for each new code. This was done to obtain consistency between the three identical tests within the 96 well-plate. Reducing the size of the standard deviation shortened the length of the error bars. This affected the overlapping regions of the error between the control and analyte values. When all three tests within the plate produced identical codes, the resulting code and standard deviation

were reported as the average code the analyte.

The consistency of the three tests on the plate was also analyzed using a similar technique. Identical wells (e.g., well containing DC1, TRIS buffer and analyte) in each of the three tests were compared using standard deviation values. If the corresponding error bars overlapped, it was determined the tests yielded identical results and were therefore consistent. If the error bars did not overlap, it was determined that the tests were not identical. If this was the result, the suspect test was not used in code determination. **Table 3** shows the process of code determination using the Masking Method.

Table 3. Codes determined **by color changes (CC)** for a plate containing three tests of caffeine. As the standard deviation factor **(SDF)** was decreased, the three tests converged to a consistent code, with no discrepancies between the three tests.

SDF	Test 1	CC	Test 2	CC	Test 3	CC	Code Differe
1.0	01-11-00-00-00-	4	01-11-00-00-00-	5	00-11-00-00-00-	4	2
0.9	01-11-00-00-00-	5	01-11-00-00-00-	5	00-11-00-00-00-	4	1
0.8	11-11-00-00-00-	6	11-11-00-00-00-	6	00-11-00-00-00-	4	2
0.7	11-11-00-00-00-	6	11-11-00-00-00-	6	10-11-00-00-00-	5	1
0.6	11-11-00-00-00-	6	11-11-00-00-00-	6	11-11-00-00-00-	6	0

4.3 Macro Method

This method resulted as an adaptation of the Masking Method. Although thorough and consistent, code determination was quite time intensive. To alleviate this, two method parameters were changed. First, a macro was designed to analyze red, green and blue values separately, as opposed to an average of all three. Second, this macro was also designed to determine the code immediately following RGB measurement. These two improvements alone dramatically decreased analysis time.

Preliminary results using this image analysis technique show improved consistency versus the previous method using human eyesight as a detection method. Studies to determine the reproducibility of this image analysis technique resulted in an average code for each analyte tested. This code was compared against all obtained codes to determine an error percentage. The average codes for three different analytes, along with their associated error percentage, can be seen in **Table 4.**

Table 4. Average codes and the associated error percentages for three of the tested analytes.

ANALYTE	CONCENTRATION	AVERAGE CODE	ERROR
Caffeine	62.5 mM	11-11-11-00-11-00-11-00	7.16%
Cocaine	62.5 mM	11-11-11-11-11-11-00-00	4.06%
Nicotine	62.5 mM	11-11-11-11-00-00-00-00	9.75%

5. DISCUSSION

Originally, visual interpretation of DETECHIP® was used to generate a 32-digit binary code exclusive to a particular analyte. While successful, this method of analysis was not only time, labor, and personnel intensive, but also was also subjective to differences in human vision.

Therefore, four new methods of image analysis were developed for more objective code determination. The photo analysis method produced codes that were generally in good agreement with those obtained from visual determination. However, the photos used for this method suffered of parallax and well shading, which had a negative effect on consistency. To overcome this problem, the plates were scanned using a flatbed scanner to create clearer images with less variability between tests.

The scanned images were subject to analysis by the remaining three methods. Image subtraction gave qualitative responses for color change, but some subjective interpretation was still required. If, after subtraction, a well was neither completely white nor completely saturated with color, it must be determined what degree of color change corresponds to a value of '1' in the code. This undesirable quality provided the path to the Masking Method. This method provided a quantitative aspect to image subtraction – providing standard deviation values that could be compared between control and analyte wells. Although consistent codes were obtained, large amounts of analysis time were required.

The Macro Method took aspects of all the previous methods to produce a simple automated analysis technique with incorporated code determination. This method separately analyzed red, green and blue values before assigning a '1' or a '0' value, which was advantageous if one color value increased while a second decreased in a similar fashion. This type of change would is not evident in the average RGB value, and would not be represented in the code determined by the Masking Method. The macro, used within ImageJ, can be modified by setting a color threshold value, altering the size of color change needed to produce a '1' in the code. This quality will be further explored in association with analyte concentration.

Through the analysis of several image collection formats and image analysis techniques, it was determined that scanned images in conjunction with the in-house designed macro for use with ImageJ software provided the most consistent means for DETECHIP® code determination. Furthermore, scanning the DETECHIP® well plates avoids problems associated with photographing the plates, such as parallax and shading around the edges of the wells. These scanned images provide a clear representation of the color in each well, while the macro allows for quantitative determination of color changes between analyte and control wells with consistency that far exceeds the other methods of analysis.

In conclusion, the advantage and value of the DETECHIP® array lies within its ability to detect and identify a multitude of chemicals spanning several different chemical classes, including abused narcotics: narcotics with cutting agents; over the counter medications; explosives and the starting materials or intermediates used to make them; pesticides and other environmental contaminants; metabolites of microorganisms; poisons; etc. We have shown that these analytes of interest can be detected not only by visual inspection of DETECHIP® but also by image analysis, making the detection technique less subjective and more user-friendly. Digital analysis also opens the door for miniaturization and automation of the DETECHIP® technology.

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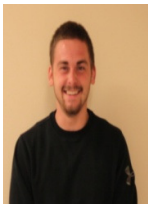

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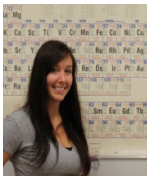

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