

DEEP COLORIMETRIC OLFACTORY-BARCODING

Cross-Reference to Related Application

[0001] This application claims the benefit of priority of Singapore Patent Application
5 No. 10202009920S, filed 6 October 2020, the content of it being hereby incorporated
by reference in its entirety for all purposes.

Technical Field

[0002] The present disclosure relates to a colorimetric barcode for olfactory detection
10 of one or more scent molecules emitted from a sample. The present disclosure also
relates to a method of forming the colorimetric barcode and uses of the colorimetric
barcode.

Background

15 [0003] The mammalian olfactory system may accurately discriminate trillions of scents
despite having only hundreds of olfactory receptors. These receptors tend to have an
affinity for a range of scent molecules. One scent molecule may bind to different
receptors with varying affinities and activate several receptors at once, forming a
complex scent fingerprint that may be carried to the central nervous system (CNS) for
20 analysis (**FIG. 1A**). It is this integration of olfactory receptor combinatorics with CNS
pattern recognition that allows the olfactory system to discriminate scents efficiently.

[0004] Mimicking the olfactory system for accurate, portable and real-time artificial
scent screening may require two crucial and inseparable components: cross-reactive
sensing and fingerprint pattern recognition. Inspired by nature, artificial scent screening
25 systems resembling the mammalian system (known as e-noses, opto-noses for olfactory,
and e-tongue for taste) have been developed for disease diagnosis and detection of
environmental contaminants, explosives, food, and drugs. Cross-reactive sensing in
these artificial systems may be achieved using cross-reactive metal oxide or
colorimetric sensor array(s) that interacts differentially with target molecules to
30 generate a fingerprint pattern. Traditional statistical methods such as linear discriminant
analysis (LDA), principal component analysis (PCA) and hierarchical cluster analysis
(HCA) may typically be used to analyze the fingerprints.

[0005] While e-noses may have been extensively researched, few may have been commercialized because they suffer from either sensing or pattern-recognition issues. For example, metal oxide-based e-noses generally require high temperatures to function and depend on cumbersome power supply and wiring. Furthermore, these sensors tend to be sensitive to humidity and unable to distinguish closely similar compounds. On the other hand, while colorimetric sensors (or opto-noses) may be highly cross-reactive and perform well at room temperature, they produce 3^n (where 3 represents red, green, blue (RGB) and n is number of sensors) non-linear, multi-dimensional datasets that tend to be inaccurately extracted and analyzed using statistical methods such as PCA, LDA, or HCA that are also time consuming. Moreover, in one reported example of a colorimetric sensor array, image pretreatment was necessary where users had to additionally calculate gas-induced RGB difference of sensors to produce a “difference map”.

[0006] There is thus a need to provide for a solution that addresses one or more of the limitations mentioned above. The solution should at least provide for olfactory detection of one or more scent molecules emitted from a sample. The solution may preferably have both a robust cross-reactive sensor array and a data analysis method that can extract information from non-linear datasets and accurately predict fingerprint patterns.

20 Summary

[0007] In a first aspect, there is provided for a colorimetric barcode for olfactory detection of one or more scent molecules emitted from a sample, the colorimetric barcode includes:

- a polymeric substrate;
- porous nanocomposites patterned as a barcode on the polymeric substrate,
- wherein each of the porous nanocomposites is patterned as a bar of the barcode,
- wherein each of the porous nanocomposites includes a matrix and a dye-containing carrier embedded in the matrix, and
- wherein each bar of the barcode includes one dye-containing carrier which is different from another dye-containing carrier in each of the other bars.

[0008] In another aspect, there is provided for a method of forming the colorimetric barcode described in various embodiments of the first aspect, the method includes:

- (i) providing a dye-containing carrier;
- (ii) mixing the dye-containing carrier with a matrix solution to form a porous nanocomposite, wherein the porous nanocomposite includes a matrix having the dye-containing carrier embedded in the matrix;
- (iii) repeating steps (i) and (ii) to form porous nanocomposites, wherein each of the porous nanocomposites includes one dye-containing carrier which is different from another dye-containing carrier in each of the other porous nanocomposites; and
- (iv) depositing the porous nanocomposites on a polymeric substrate to form bars of the colorimetric barcode.

[0009] In another aspect, there is provided for a method of identifying freshness of food using the colorimetric barcode described in various embodiments of the first aspect, the method includes:

providing a training dataset comprising training image data that includes training pairs of the colorimetric barcode described in various embodiments of the first aspect and a corresponding freshness of the food; and

training a neural network with the training dataset to obtain the trained neural network.

[0010] In another aspect, there is provided for a non-transitory computer-readable medium storing computer executable code that includes instructions for identifying freshness of food using the colorimetric barcode described in various embodiments of the first aspect.

[0011] In another aspect, there is provided for a computer executable code that includes instructions for identifying freshness of food using the colorimetric barcode described in various embodiments of the first aspect.

Brief Description of the Drawings

[0012] The drawings are not necessarily to scale, emphasis instead generally being placed upon illustrating the principles of the present disclosure. In the following description, various embodiments of the present disclosure are described with reference to the following drawings, in which:

[0013] FIG. 1A depicts a working principle of the human olfactory system. In the human olfactory system, volatile amines from meat activate several odor-binding

proteins of the olfactory cell (left) and trigger electrochemical potentials that travel to the brain center via olfactory nerve fibers (middle). Tapping on prior knowledge, the brain analyses the action potential patterns and identifies them as different levels of meat freshness (right).

5 **[0014]** FIG. 1B depicts a system based on colorimetric barcode combinatorics and (deep convolutional neural networks) DCNN of the present disclosure. In the system, volatile amines from meat interact with sensors in the barcode and trigger a color change based on halochromism. Colorimetric barcode image showing barcode combinatorics is captured and wirelessly uploaded to the cloud via a smartphone APP.

10 Using a trained DCNN, meat freshness can be displayed on the smartphone within 30 seconds. Advantageously, the present deep colorimetric olfactory-barcoding is deemed a system-level mimicry of olfactory system with automatization and high accuracy. The mimicry of receptor combinatorics is realized by the strip combinatorics to provide scent fingerprint where scent triggers the color changes of interactive strip sensors

15 based on halochromism principle. The mimicry of human CNS is the DCNN with learnable feature extractor and classifier specific which could recognize scent fingerprint and identify scent information. The integration of barcode and DCNN via a software on a smartphone provides a platform that could automatically identify unknown samples within 30 seconds by scanning the barcode. This is advantageous

20 compared to complicated electronic wire connection for electronic nose (e-nose) and time consuming statistical methods in optoelectronic nose.

[0015] FIG. 2A is a schematic showing the fabrication of chitosan nanoparticles-dye/cellulose acetate (CNPs-Dye/CA). Dyes (denoted by the stars) are cross-linked with chitosan (denoted by the lines) using sodium tripolyphosphate (TPP, denoted by the cross) according to an ionic gelation method. The CNPs-Dye is embedded on cellulose acetate (CA) matrix to form CNPs-Dye/CA. The inset in middle of the image depicts a non-limiting example of the chemical structure of a CNP-Dye. The dye used in this example is tetraiodophenolsulfonephthalein (TET). Strong intramolecular forces between positively charged protonated amine groups ($-\text{NH}_3^+$) of chitosan and

25 negatively charged deprotonated hydroxyl groups ($-\text{O}^-$) in the dye prevent dye leakage.

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[0016] FIG. 2B is a schematic showing barcode patterning on PDMS. Oxygen plasma processing (30%, 2 mins) using a shadow mask forms hydrophilic patterns on PDMS.

Drop-casting of CNPs-Dye/CA solution (1 μ L) on the plasma-treated patterns forms the 20-bar colorimetric barcode. Each bar has a different CNPs-Dye/CA solution. Barcode size is 1 mm $\times$ 0.6 mm and gap between neighbouring bar is 1 mm.

[0017] FIG. 2C shows in the top row scanning electron microscopy (SEM) images of chitosan nanoparticles (CNP), aggregated chitosan nanoparticles with TET (CNP-TET), and cellulose acetate (CA) in the left, center and right, respectively. The right image shows that CA fibers have a highly porous network structure which is good for gas transferring. The inset in the right image shows that CA fibers without nanoparticles loading are smoother compared to CNP-TET/CA. In the left and center images, the scale bars denote 200 nm. In the right image, scale bar denotes 1 μ m and scale bar in inset denotes 400 nm. FIG. 2C in the bottom row shows a SEM image of CNPs-Dye/CA, demonstrating the composite is highly porous. The inset shows CNP were immobilized on the surface of CA fibers. Scale bar denotes 1 μ m and scale bar of inset denotes 400 nm.

[0018] FIG. 2D shows dye leakage test result for different films, wherein CNPs-TET and CNPs-TET/CA films had negligible dye leakage. Films were soaked in water for different durations and absorbance of the supernatant were measured at 435 nm. Data are representative of three independent experiments. The error bars are the standard deviation from three independent experiments.

[0019] FIG. 2E shows photographs of CA/TET and CNPs-TET/CA (1:4) films soaked in water for 84 hrs in the top and bottom images, respectively. Dye leaked from CA/TET but not CNPs-TET/CA film.

[0020] FIG. 2F is a plot of sensing performance of different colorimetric strips exposed to 20 ppm methylamine (MA) gas. Strip response is described by measuring Euclidean distance (ED) of RGB values of the barcode before and after exposure to MA. Curve linking the ED values of different strips is for guiding the eye. Optimal sensing materials of CNPs-Dye/CA (1:4) was selected for barcode preparation. The error bars are the standard deviation of three independent experiments.

[0021] FIG. 3A shows the DCNN for barcode recognition. The left image shows the dataset contained 3475 images for DCNN training and 686 images for model testing. DCNN based on the ResNet 101 backbone was used for food freshness classification (right). The right image shows the DCNN architecture has input, multiple convolution

(conv.), full connection (FC), and output layers. When the barcode image is input into the network, the predicted freshness category (circles denoted as the output) for the test sample is given.

[0022] FIG. 3B is a plot of training accuracy of DCNN increases as epochs (number of passes the algorithm completes through the entire dataset) increase. Training loss represents the difference between predicted and actual result.

[0023] FIG. 3C is a comparison of detection accuracy rate for chicken, fish, and beef freshness based on Euclidean distance calculations and DCNN training. DCNN had an overall higher accuracy rate (98.5%) than ED (61.7%).

[0024] FIG. 3D shows a confusion matrix for the 686 test images showing DCNN accurately classified the images into the three freshness categories. Only ten images were wrongly classified.

[0025] FIG. 3E shows photographs of smartphone interfaces displaying the state of the meat as either fresh (left image), less fresh (middle image), or spoiled (right image) after scanning the barcode.

[0026] FIG. 3F shows a schematic diagram (left image) and digital image of fully packaged meat with sensors integrated in the inner side of PVC film (right image).

[0027] FIG. 4A is a table showing the list of biocompatible chromophore dyes on the colorimetric barcode. Each of the 20 different dyes responds within a different pH range, covering the detection range of gases emitted from rotting meats. When different gases are present in varying concentrations, each bar of the barcode displays a different color or range of coloration.

[0028] FIG. 4B shows a bioamine sensing mechanism based on deprotonation-induced dye color changes, particularly the sensing mechanism of amine gases to the present barcode (top image). The bottom image shows an acid-base reaction between ammonia (NH_3) and bromothymol blue (BPB) (the pH indicator wrapped in the first strip of barcode), wherein the color dye changes its color reversibly from yellow (protonated form) to blue (deprotonated form).

[0029] FIG. 5 shows original and RGB (red, green, blue) images of colorimetric barcode for methylamine (MA) gas sensing. Increasing concentrations of MA turned the barcode on the strip from yellow to deep blue. The RGB images were produced by

extracting R, G, and B values from the corresponding strips of original images and were used for Euclidean Distance calculation.

[0030] FIG. 6 shows the response of colorimetric strip to methylamine (MA) with different concentration by using Tetraiodophenolsulfonephthalein (TET) as a model dye. The Euclidean distances of the barcode before and after exposure to MA increase with MA concentration increasing, indicating that MA activated the chromophore and the barcode functioned as a scent sensor. Error bar are standard deviation of three parallel experiments.

[0031] FIG. 7A shows scanned images (Canon scanner, LBP7010C, 600 dpi) of barcode exposed to ammonia (NH₃), MA, trimethylamine (TMA), putrescine (PUT) and cadaverine (CAD) having different gases display different bar combinatorics. Barcode color changes as gas concentration increased from 0.20 to 100.0 ppm.

[0032] FIG. 7B is a plot of Euclidean distance of R, G, and B values in the different barcodes increase after exposure to higher concentrations of amine gas. The error bars are the standard deviation from eight independent experiments.

[0033] FIG. 7C shows a boxplot of the distribution of ED values for NH₃, MA, TMA, CAD, and PUT at 20 ppm ($n = 8$), which shows that these five amine gases can be clearly distinguished at 20 ppm. *** represents $p < 0.001$ (t -test).

[0034] FIG. 7D shows a cluster tree produced by hierarchical cluster analysis, which shows that the five amine gases can be distinguished between 20 and 100 ppm.

[0035] FIG. 7E shows RGB images of colorimetric barcode when exposed to NH₃, MA, TMA, PUT and CAD with concentrations varied from 0 ppm to 100.00 ppm. The RGB images were obtained by extracting R, G, and B values of scanning images in FIG. 7C and were used for Euclidean distance value calculation.

[0036] FIG. 8 is principal component analysis (PCA) score plot, which shows that 6 principal components (PCs) are needed to reach 90% of total variance, 8 PCs needed for reach 95% of total variance, and 10 PCs needed for reach 99% of total variance.

[0037] FIG. 9 is a plot of clusters of 5 amine gases via 3D PCA score plot. The three dimensions plotted only encompass 76.88% of the total variance, shows that clusters of NH₃, MA, TMA, CAD and PUT can be clearly separated from 20 to 100 ppm.

[0038] FIG. 10 shows building barcode dataset for DCNN training and testing. Colorimetric barcodes that adhere to the packages of meat gradually change the color

with the increase of the meat storage intervals. These images collected at 0 to 48 hrs were used for DCNN training and testing.

[0039] FIG. 11 shows a scheme for Conway dish method for TVB-N measurement. In detail, 10 g minced meat sample and 100 mL of distilled water were homogenized. 30 mins later, the fluid was filtered to obtain a sample liquid. Then, a 3 mL boric acid absorbing solution (20 g/L) and a 50 μ L pH indicator (methyl red: bromocresol green = 1:5, V/V) were added in the inner chamber of the Conway dish. In the outer chamber, 3 mL potassium carbonate saturated solution and 1 mL sample liquid were added in sequence. The Conway dish was kept sealed and maintained at 37°C for 2 hrs. The volatile amine gases were absorbed by boric acid absorbing solution. Using 0.1 M hydrochloric acid to titrate, the amount of volatile amines can be calculated by 0.1 M hydrochloric acid usage.

[0040] FIG. 12 shows the subconfusion matrix for the freshness classifier of chicken, fish and beef samples. For chicken, all testing images (102 images of fresh, 54 images of less fresh, and 112 images of spoiled) were classified with the classification accuracy 99.0% of fresh, 96.3% of less fresh and 100% of spoiled, respectively. For fish, 52 images of fresh, 80 images of less fresh, and 74 images of spoiled were also classified with the classification accuracy 98.1% of fresh, 98.8% of less fresh and 100% of spoiled, respectively. For beef, all images (97 images of fresh, 86 images of less fresh, and 29 images of spoiled) were classified with the classification accuracy 95.9% of fresh, 98.8% of less fresh and 100% of spoiled, respectively.

[0041] FIG. 13 shows classification of the Euclidean distance (ED) values to three freshness levels. Both the ED (black line) and TVB-N (red line) value of each sample were obtained. Left, middle, and right images denote for chicken, fish, beef, respectively. The freshness of each meat samples with different storage intervals based on TVB-N values was first labeled. According to the freshness of each sample, the ED value ranges corresponding to freshness level are obtained. These ED value ranges are used for predicting freshness state of other samples. Error bar of ED curve are standard deviation of eight parallel experiment and error bar of TVB-N curve are standard deviation of three parallel experiment.

[0042] FIG. 14A shows the Euclidean distance distribution of chicken. A total of 60 images were used and 20 images for each freshness state were used. ED values of fresh and less fresh category were all not significantly different ($p > 0.05$).

[0043] FIG. 14B shows the Euclidean distance distribution of fish. A total of 60 images were used and 20 images for each freshness state were used. ED values of fresh and less fresh category were all not significantly different ($p > 0.05$).

[0044] FIG. 14C shows the Euclidean distance distribution of beef. A total of 60 images for were used and 20 images for each freshness state were used. ED values of fresh and less fresh category were all not significantly different ($p > 0.05$).

[0045] FIG. 15A shows the barcoding system for accurate freshness prediction of fish stored at 4°C, wherein the barcode images are collected at different storage intervals.

[0046] FIG. 15B shows a dataset containing 112 images (8 sensors x 2 photos x 7 time points) for model testing (left image). Images are uploaded for freshness prediction (middle image). Confusion matrix for the 112 test images showed that DCNN accurately classified the images into the three freshness categories with overall accuracy of 92.0% (right image). Only 9 images were wrongly classified.

[0047] FIG. 16 shows classification of the fish meat stored at 4°C to three freshness levels. Based on the TVB-N value, the freshness of each sample with different storage intervals was labeled. Error bars of TVB-N curve are standard deviation of three parallel experiment.

[0048] FIG. 17 is a table showing comparison between the present system and reported works relating to artificial scent screening systems.

Detailed Description

[0049] The following detailed description refers to the accompanying drawings that show, by way of illustration, specific details and embodiments in which the present disclosure may be practised.

[0050] Features that are described in the context of an embodiment may correspondingly be applicable to the same or similar features in the other embodiments.

Features that are described in the context of an embodiment may correspondingly be applicable to the other embodiments, even if not explicitly described in these other embodiments. Furthermore, additions and/or combinations and/or alternatives as

described for a feature in the context of an embodiment may correspondingly be applicable to the same or similar feature in the other embodiments.

[0051] Mammal olfactory is deemed an efficient scent discrimination system, which may consist of only hundreds of olfactory receptors but yet able to distinguish more than 1 trillion olfactory stimuli with high sensitivity. The integration of olfactory receptor combinatorics with central neuron system pattern recognition contributes to the high scent discrimination efficiency of olfactory system. The receptor combinatorics may have inspired electronic or optoelectronic nose based on cross-reactive sensor arrays to substitute traditional lock-and-key sensing modality, which may have shown wide applications in disease diagnosis, environmental safety, public security, and food safety. The systematic integration tends to be moving towards pint-sized, portable, wearable, and even implantables for human intelligent life. However, while the large amount of data may enhance discrimination efficiency among similar analytes, it poses great burden for time-consuming statistical methods such as hierarchical cluster analysis (HCA), linear discriminant analysis (LDA), and principal component analysis (PCA). Particularly, as most traditional analytical methods tend to be inherently linear, poor prediction accuracy may often be observed in case where the data has a non-linear correlation between dimensions. To bridge such gap, it is necessary to develop a system-level mimicry of olfactory system to provide automatic, real-time, and high accurate scent discrimination platform.

[0052] The present disclosure relates to a colorimetric barcode and a system including the colorimetric barcode that addresses one or more of the limitations mentioned above. Details of various embodiments of the present colorimetric barcode, method of forming the colorimetric barcode, and its uses thereof, and advantages associated with the various embodiments are now described below.

[0053] In the present disclosure, there is provided a colorimetric barcode for olfactory detection of one or more scent molecules emitted from a sample. The term “colorimetric barcode” is interchangeably used with “colorimetric sensor array”, as the colorimetric barcode herein may include a pair or more parallel lines arranged in an array optionally having varying widths and varying spacings between the parallel lines. For brevity, the colorimetric barcode may be referred herein as “barcode”.

[0054] The colorimetric barcode may include a polymeric substrate. The colorimetric barcode may include porous nanocomposites patterned as a barcode on the polymeric substrate, wherein each of the porous nanocomposites is patterned as a bar of the barcode, wherein each of the porous nanocomposites includes a matrix and a dye-containing carrier embedded in the matrix, and wherein each bar of the barcode includes one dye-containing carrier which is different from another dye-containing carrier in each of the other bars. The dye-containing carrier may be referred herein to as “nanoparticle carrier” as it can be a nanoparticle. For brevity, the dye-containing carrier may be referred herein to as “dye carrier”. A non-limiting example of the porous nanocomposites is depicted in rightmost image of FIG. 2A.

[0055] In various embodiments, the dye-containing carrier may include a carrier formed from a polymer. The polymer may be a nanoparticle, i.e. the carrier may be a nanoparticle. The polymer may be cross-linked with a dye (e.g. a vapochromic dye) and as such referred to as the dye-containing carrier. A vapochromic dye is a dye that changes colour in response to a vapor of a compound, such as vapor of an organic compound.

[0056] The carrier (i.e. the polymer) and the dye may each have a functional group that forms interaction with each other to avoid dye leakage. The interaction may be ionic bond, encapsulation, π - π interaction, van der Waals bond, covalent bonding, or electrostatic interaction. For example, if the dye has a hydroxyl group, the carrier may have an amine functional group, which then leads to strong intra-molecular forces with the deprotonated hydroxyl group of the dye. Dyes with carboxyl group may also form interaction with the carrier having amine functional group.

[0057] In various non-limiting embodiments, the polymer may have an amine functional group. In various embodiments, the polymer may include chitosan, acrylamide copolymerized 2-(dimethylamino)ethyl methacrylate, polydiacetylene, or polyaniline.

[0058] In various embodiments, the vapochromic dye may include a halochromic dye and/or a metalloporphyrin. A halochromic dye is a dye that changes color in response to a change in pH. In various embodiments, the vapochromic dye may include (i) a deprotonated hydroxyl group or (ii) a deprotonated carboxyl group, which interacts with the polymer to have the vapochromic dye confined within the matrix. Understandably,

referring to FIG. 2A as a non-limiting example, the vapochromic dye is first attached to the polymer to form the dye-containing carrier, which is then mixed with the matrix (e.g. cellulose acetate). As the carrier (e.g. polymeric particles) gets trapped in the matrix due to the matrix's network structure, the vapochromic dye becomes confined therein as it is already attached to the carrier. In various embodiments, the vapochromic dye may include bromothymol blue, bromocresol green, methyl red, curcumin, alpha-naphtholphthalein, nitrazine yellow, Nile blue, bromocresol purple, alizarin red S, alizarin, chlorophenol red, rosolic acid, fluorescein, m-cresol purple, thymol blue, cresol red, p-naphtholbenzein, brilliant yellow, pyrocatechol violet, and/or tetraiodophenolsulfonephthalein.

[0059] The colorimetric barcode may further include a cross-linker for forming the carrier from the polymer. The cross-linker may be a salt of a polyanion. The cross-linker may include tripolyphosphate or glutaraldehyde. The tripolyphosphate may include sodium tripolyphosphate (TPP), which is a salt of a polyanion. The cross-linker is to cross-link, for example, the polymer such as chitosan to form the carrier, as shown in the inset under the center image of FIG. 2A, wherein TPP is illustrated as cross-linking chains of chitosan. In various embodiments, the cross-linker may have a functional group that can interact with the carrier. For example, the cross-linker may have the same functional group as the dye, which also interacts with the carrier. The interactions between the carrier and dye may be ionic bond, encapsulation, π - π interaction, van der Waals bond, electrostatic interaction, or covalent bonding. In several non-limiting examples, TPP may be used as a cross-linker for dyes containing -NH₂ functional group and the cross-linking effect is based on ionic interaction. Glutaraldehyde may also be used as a cross-linker for dyes containing -NH₂ functional group and the cross-linking effect is based on covalent interaction.

[0060] In various embodiments, the matrix provides a 3D porous structure for housing the dye-containing carrier. As the matrix has a 3D porous structure, it provides abundant gas sensing sites, i.e. the 3D porous structure provides several openings, rendering it accessible for scent molecules to easily interact with the dyes housed in the matrix. At the same time, the 3D porous matrix may also help in trapping the dyes therein, preventing dye from leaking easily. In various non-limiting embodiments, the matrix may include a cellulose-based material. The cellulose-based material may include

5 methyl cellulose, ethyl cellulose, hydroxyethyl cellulose, and/or other cellulose derivatives with similar average molecular weight range. Cellulose derivatives with such average molecular weight range are breathable, easy to decompose and water-resistant. A non-limiting example of the cellulose-based matrix may include cellulose acetate. The cellulose acetate may have an average molecular weight in the range of 20000 Da to 50000 Da.

10 **[0061]** In several non-limiting examples, the porous nanocomposite may be a 3D porous chitosan nanoparticles (CNPs)-dye/cellulose acetate (CA) composite. In such instances, the carrier is the chitosan nanoparticle and the porous 3D matrix is cellulose acetate. The chitosan may have a viscosity of 200 cP to 800 cP (e.g. 1 wt. % in 1% acetic acid (25°C)). This allows formation of a suitable solution system for CNPs-Dye preparation. Advantageously, the chitosan nanoparticles are biodegradable and have high specific surface area.

15 **[0062]** In various embodiments, the polymeric substrate may be moisture-resistant and transparent. The polymeric substrate may include or may be formed of poly(dimethylsiloxane), polyvinyl pyrrolidone, thermoplastic polyurethane, a polyolefin elastomer, or polyethylene terephthalate. The polymeric substrate may be subject to a treatment that renders it hydrophilic for the porous nanocomposites to be patterned thereon. In various non-limiting embodiments, the number of bars forming the colorimetric barcode may range from, for example, 10 to 36. In other words, there can be 10 to 36 different porous nanocomposites each patterned as a bar on the polymeric substrate to form the barcode. The number of porous nanocomposites and hence the number of bars forming the barcode is not limited to such a range, and can be more or less.

20 **[0063]** In summary, the colorimetric barcode (i.e. colorimetric sensor array) may include at least one porous nanocomposite formed on a patterned polymer film, and wherein each porous nanocomposite may include a dye cross-linked with a carrier embedded in and/or on a porous three-dimensional (3D) matrix.

25 **[0064]** The present disclosure provides for a method of forming the colorimetric barcode described in various embodiments of the first aspect. Embodiments and advantages described for the colorimetric barcode of the first aspect can be analogously valid for the present method described herein, and vice versa. As the various

embodiments and advantages have already been described above and in the examples hereinbelow, they shall not be iterated for brevity. For understanding of the present method, examples 2A and 2B hereinbelow can be referred to, wherein examples 2A and 2B are meant to be non-limiting demonstration of the present method.

5 **[0065]** The method may include (i) providing a dye-containing carrier, (ii) mixing the dye-containing carrier with a matrix solution to form a porous nanocomposite, wherein the porous nanocomposite may include a matrix having the dye-containing carrier embedded in the matrix, repeating steps (i) and (ii) to form porous nanocomposites, wherein each of the porous nanocomposites may include one dye-containing carrier
10 (e.g. one carrier having a vapochromic dye) which is different from another dye-containing carrier (e.g. the same or different carrier having a different vapochromic dye) in each of the other porous nanocomposites, and (iv) depositing the porous nanocomposites on a polymeric substrate to form bars of the colorimetric barcode. In other words, each of the porous nanocomposites contains a different dye and hence a
15 different dye-containing carrier. Understandably, each bar of the barcode then includes one dye-containing carrier which is different from another dye-containing carrier in each of the other bars.

[0066] In various embodiments, providing the dye-containing carrier may include mixing a dye (e.g. vapochromic dye) with a polymer. In various embodiments,
20 providing the dye-containing carrier may further include mixing the dye (e.g. vapochromic dye) with the polymer in the presence of a cross-linker. Various embodiments of the dye, vapochromic dye and polymer are already described above and shall not be iterated for brevity.

[0067] In various embodiments, depositing the porous nanocomposites on the
25 polymeric substrate may include forming trenches in the polymeric substrate, and depositing the porous nanocomposites into the trenches to form the bars of the colorimetric barcode. As mentioned above, each bar of the barcode may include one dye-containing carrier which is different from another dye-containing carrier in each of the other bars. Various embodiments of the polymeric substrate are already described
30 above and shall not be iterated for brevity.

[0068] In various embodiments, depositing the porous nanocomposites on the polymeric substrate may further include rendering the polymeric substrate hydrophilic by exposing the trenches to oxygen plasma treatment.

[0069] In summary, the present method may be summarized as a method of forming the colorimetric sensor array, which may include adding a cross-linker to a solution of carrier (i.e. the polymer) and dye to form a dye composite (i.e. dye-containing carrier), separating the dye composite from the solution, and re-suspending the dye composite in a solution of three-dimensional (3D) matrix to form a dye composite/matrix solution (i.e. porous nanocomposite solution), and depositing the dye composite/matrix solution on a patterned polymer film to form the colorimetric sensor array. In certain instances, as the formed dye composite may be in the form of a gel, the present method may be deemed an ionic gelation method in such instances. The ionic gelation method is just a non-limiting example of the present method as other cross-linkers, which may not interact via ionic interaction or which may not lead to gel formation, may be used.

[0070] In various embodiments, additives may be further added to facilitate the dissolution of the carrier. For example, additives may be added to acidify the solution for better dissolution of chitosan. An example of such additive may include acetate and sodium acetate as a buffer to tune the pH of solution.

[0071] The present disclosure further provides for a method of identifying freshness of food using the colorimetric barcode described in various embodiments of the first aspect. Embodiments and advantages described for the colorimetric barcode of the first aspect can be analogously valid for the present method described herein, and vice versa. As the various embodiments and advantages have already been described above and in the examples hereinbelow, they shall not be iterated for brevity.

[0072] The method may include providing a training dataset that includes training image data having training pairs of the colorimetric barcode described in various embodiments of the first aspect and a corresponding freshness of the food, and training a neural network with the training dataset to obtain the trained neural network.

[0073] In various embodiments, the method may further include identifying the freshness of the food using the trained neural network.

[0074] In various embodiments, the trained neural network may be a deep convolutional neural network (DCNN).

[0075] The present disclosure further provides for a non-transitory computer-readable medium storing computer executable code that includes instructions for identifying freshness of food using the colorimetric barcode described in various embodiments of the first aspect. Embodiments and advantages described for the colorimetric barcode of the first aspect can be analogously valid for the present non-transitory computer-readable medium storing computer executable code described herein, and vice versa. As the various embodiments and advantages have already been described above and in the examples hereinbelow, they shall not be iterated for brevity.

[0076] The present disclosure further provides for a computer executable code that includes instructions for identifying freshness of food using the colorimetric barcode described in various embodiments of the first aspect. Embodiments and advantages described for the colorimetric barcode of the first aspect can be analogously valid for the present computer executable code described herein, and vice versa. As the various embodiments and advantages have already been described above and in the examples hereinbelow, they shall not be iterated for brevity.

[0077] Said differently, in summary, the present colorimetric barcode may be used in a method to monitor meat freshness, and configured in a meat freshness monitoring system.

[0078] The method of monitoring the meat freshness may include collecting an image of the colorimetric sensory array that has been exposed to a meat, providing the taken image as an input to a trained learning model and obtaining a meat freshness status, wherein the trained learning model has been trained through machine learning to gauge freshness of the meat from optical properties of the colorimetric sensor array.

[0079] The trained learning model may include at least three-class classification model (fresh, less fresh and spoilt). The optical properties of the colorimetric sensor array may include colours and/or intensity of colours.

[0080] The meat freshness monitoring system may include an imaging device that obtains an image of the colorimetric sensory array of which is exposed to a meat, and a meat freshness analysis or determination unit that receives the taken image as an input to a trained learning model and obtains a meat freshness status, wherein the trained learning model has been trained through machine learning to gauge meat freshness from optical properties of the colorimetric sensor array. The imaging device may be any

visible camera. The meat freshness analysis or determination unit may be a software or an interface design of a software in a phone application. The trained learning model may include at least three-class classification model (fresh, less fresh and spoilt). The optical properties of the colorimetric sensor array may include colours and/or intensity of colours.

[0081] In a non-limiting example, a deep colorimetric olfactory-barcoding integrated system includes a human olfactory mimic-system that combines the present colorimetric sensor array with DCNNs, a training and identification colorimetric sensor array images using DCNN technology, and a smartphone application interface configured and operable for classification results display, wherein the trained DCNN is incorporated in the smartphone application.

[0082] The word “substantially” does not exclude “completely” e.g. a composition which is “substantially free” from Y may be completely free from Y. Where necessary, the word “substantially” may be omitted from the definition of the present disclosure.

[0083] In the context of various embodiments, the articles “a”, “an” and “the” as used with regard to a feature or element include a reference to one or more of the features or elements.

[0084] In the context of various embodiments, the term “about” or “approximately” as applied to a numeric value encompasses the exact value and a reasonable variance.

[0085] As used herein, the term “and/or” includes any and all combinations of one or more of the associated listed items.

[0086] Unless specified otherwise, the terms "comprising" and "comprise", and grammatical variants thereof, are intended to represent "open" or "inclusive" language such that they include recited elements but also permit inclusion of additional, unrecited elements.

Examples

[0087] The present disclosure relates to a colorimetric barcode configurable in an artificial scent screening system (i.e. deep colorimetric olfactory-barcoding) that is portable, operably automatic, accurate, and works on real-time. The system may also involve deep learning methods, especially deep convolutional neural networks (DCNN). Deep learning methods, e.g. DCNN, with learnable feature extractor and classifier have

been incorporated with features of automatic features extraction, high accuracy, high efficiency, which are successfully applied in images analysis, such as face recognition, skin cancer photos classification, etc. Herein, DCNN help solve multi-dimensional data process problems for efficient colorimetric fingerprint recognition.

5 [0088] Particularly, the present disclosure relates to a deep colorimetric olfactory-barcoding that combines cross-reactive colorimetric strips that provide scent fingerprint with DCNN for fingerprint recognition, which is a system-level mimicry of olfactory system. Said differently, use of the present colorimetric barcode with DCNN may be herein referred to as the “present system”, or for brevity, the “system”. DCNN was
10 incorporated with software on smartphone to capture barcode image and display detection results directly. The present deep colorimetric olfactory barcoding is portable, non-destructive, intelligent and serves as a real-time scent screening platform which could be directly used in food industry, public security, and even disease diagnosis.

[0089] The present colorimetric barcode, method of forming the colorimetric barcode
15 and its uses, are described in further details, by way of non-limiting examples, as set forth below.

[0090] Example 1: Materials

[0091] Chitosan (medium molecular weight), cellulose acetate (Mw = 30000 Da), ammonia hydrogen solution (25% in water), methylamine solution (33% in absolute
20 ethanol), trimethylamine solution (45% in water), cadaverine (> 95%), putrescine (> 98.5%) and 20 kinds of dyes were all purchased from Sigma-Aldrich (Singapore).

[0092] Example 2A: Synthesis of Chitosan Nanoparticles-Dye/Cellulose Acetate (CNPs-Dye/CA)

[0093] Dye encapsulated nanocomposites (i.e. porous nanocomposites) were
25 synthesized using ionic gelation method. In detail, 2.5 mL chitosan solution (5 mg/mL, 2% acetic acid (HAc)), 2.5 mL sodium acetate solution (0.2 mol/L), 0.4 mL dye solution (5 mg/mL in 2-methoxyethanol) were added in a bottle. Under stirring, 5 mL sodium tripolyphosphate (TPP) solution (5 mg/mL) was added dropwise. Thirty minutes later, the solution was centrifuged at 8000 rpm for 10 minutes. The sediment was washed
30 twice with water, then was resuspended in 4 mL cellulose acetate solution (5 mg/mL in 2-methoxyethanol). Tetraiodophenolsulfonephthalein (TET) was used as one of the

dyes. The synthesis procedures, chemical interaction and microstructure are shown in FIG. 2A.

[0094] To ensure biocompatibility, safety, and high sensitivity, the present method provides for preparation of a colorimetric sensor array. Chitosan nanoparticles (CNPs) were chosen as the carrier and cellulose acetate building (CA) blocks for constructing a 3D structure. Both CNPs and CA are non-toxic, biocompatible, biodegradable and environmentally friendly, which guarantees the biocompatibility of the present colorimetric sensor array. High dye interaction on CNPs to prevent dye leakage guarantee the testing safety, which is achieved via the intra-molecular forces between protonated ammonium ions (NH_3^+) of chitosan and negatively charged hydroxyl ions (OH^-) of the dyes. The 3D porous CNPs-Dye/CA structure has high dye preservation ability and provides abundant gas sensing sites. As such, the CNPs-Dye/CA was used to fabricate 3D porous barcodes, wherein the CNPs-Dye/CA prevents not only dye leakage, but also provide abundant gas sensing sites.

[0095] Example 2B: PDMS Film Preparation and Fabrication of Colorimetric Barcode

[0096] First, polydimethylsiloxane (PDMS) precursors were fully mixed with mass ratio of 10:1 (the silicone prepolymer: the crosslinker). Second, the mixture was poured onto a fluorinated silicon wafer under spin coating at the speed of 350 rpm for 30 seconds. After curing at 60 °C for 4 hours, PDMS was peeled off and cut into the required size.

[0097] Polydimethylsiloxane (PDMS) film with chemical inertness, moisture-resistance, and high transparency was chosen as the substrate for CNPs-Dye/CA nanocomposites. Due to small amount of CNPs-Dye/CA solution for each strip and easy preparation procedures, mass production of colorimetric barcode is facile via ink-jet printing technology.

[0098] The schematic diagram of colorimetric barcode fabrication was shown in FIG. 2B. Firstly, PDMS film was prepared as the substrate for CNPs-Dye/CA nanocomposites. Through oxygen plasma processing (30%, 2 mins) using a shadow mask, exposed area of PDMS film became hydrophilic. Then the CNPs-Dye/CA solution was dropped and dispersed quickly in the hydrophilic region within seconds. After drying at room temperature, colorimetric barcode composed of 20 kinds of CNPs-

Dye/CA strips was obtained where the strip length x width was 1 mm x 0.6 mm, and gap between neighbouring strip was 1 mm.

[0099] Example 3A: Method for Deep Colorimetric Olfactory-Barcoding and its Application on Meat Freshness Monitoring

5 [00100] Meat freshness monitoring was used herein as an application of the present deep colorimetric olfactory-barcoding. Details are described as follows.

[00101] Firstly, the colorimetric barcodes were fixed on transparent meat packages. It is noted that softness and transparency of barcode greatly benefits the facile integration with package. Meat with barcode package is stored at 25°C, and barcode images are
10 taken at different interval using a smartphone camera. The labeling of barcode is based on total volatile nitrogen (TVB-N) value using Conway dish method, which is the international standard for meat freshness. The classification of TVB-N value is according to a reported reference where meat with TVB-N value ≤ 15 mg/100 g is considered as fresh, 15 – 20 mg/100 g is edible but less fresh, and ≥ 20 mg/100 g
15 indicates inedible and spoilage. 4161 images for three kinds of meat are obtained, which constituted the dataset for meat freshness evaluation. 4161 images are randomly divided into 3475 images for model training and 686 images for validation.

[00102] A three classes classification model with ResNet 101 backbone was designed to predict three kinds of freshness state (FIG. 3A). During the training and testing, the
20 images were resized to 224 * 224 as input. Through the ResNet 101 backbone and a three dimension Softmax layer, the model predicted the estimated freshness state. At the beginning of training, the learning rate as 0.1 with 0.9 was used. The learning rate was decayed to 0.05 and 0.01 when the validation accuracy exceeds 70 and 80, respectively. 900 epochs were totally trained. Training accuracy continuously increases
25 with epochs increasing (FIG. 3B). 686 validated images were used to evaluate the DCNN accuracy. As shown in FIG. 3C, for the three kinds of meat, the images were classified with 100% accuracy for the spoiled category; in chicken, accuracy was 99.0% for fresh, 96.3% for less fresh; in fish, accuracy was 98.1% for fresh, 98.8% for less fresh; in beef, accuracy was 95.9% for fresh, 98.8% for less fresh. Besides, confusion
30 matrix is derived to further illustrate the accuracy of 686 validated images in DCNN method at category level (FIG. 3D). The overall accuracy rate of DCNN classification reached to 98.5%.

[00103] For comparison, Euclidean Distance (ED) value was also calculated for meat freshness prediction. The center of colorimetric barcode was used as reference point for white balance process to correct the light affects. ED values show gradually increasing over storage time, which is similar to TVB-N value. The responsive curves were used for unknown meat sample analyzing. Twenty colorimetric barcode images for each freshness state were randomly selected to assess the accuracy rate of ED analysis. This model achieved 35.0%, 55.0%, 100.0% for fresh, less fresh, and spoiled in chicken, respectively. Similarly, it achieved 90.0% for fresh, 30.0% for less fresh, 90.0% for spoiled in fish, and 40.0% for fresh, 35.0% for less fresh, 100.0% for spoiled in beef (FIG. 3C). The total accuracy rate of ED analysis was 61.7%, which is lower than that of DCNN classification, especially fresh and less fresh groups. To automatize the overall procedures, DCNN is incorporated in a smartphone application (APP), NTUFoodCheck, which provided a user-friendly interface for image collection, image uploading, and results display in real-time (FIG. 3E). Briefly, for meat freshness monitoring, user just opens APP to capture the colorimetric barcode image, and then meat freshness result is displayed on the screen based on the classification result of DCNN model. The total measurement time was within 30 seconds.

[00104] **Example 3B: Amine Gases Concentration Calculation**

[00105] Amine gases detection experiments were performed in a 3.0 L chamber with temperature controlled at 20 °C. The colorimetric barcode was hung in the center of chamber. The required gas concentration was reached by injecting certain volume liquid analyte into the chamber. The resulting gas concentration (C_{ppm}) was calculated using the following formula:

$$C_{ppm} = \frac{V_{\mu L} \times D_{mg/L} \times W}{M_{g/mol} \times V_L} \times 22.4 \times 10^7$$

[00106] where, $V_{\mu L}$ is liquid analyte volume, $D_{mg/L}$ is the liquid density, W is the mass fraction of the liquid, $M_{g/mol}$ is the molecular weight of the liquid analyte, V_L is the chamber volume.

[00107] **Example 3C: TVB-N Measurement**

[00108] To test TVB-N without the interference of image collection, another 24 (8 storage intervals) x 3 parallel experiments) packages of meat were prepared for TVB-

N values testing. The meat packaging procedures and selected storage intervals for TVB-N testing are the same as that for barcode. Details were as follows.

[00109] 10 g of minced meat sample and 100 mL of distilled water were homogenized. 30 mins later, the fluid was filtered to obtain a sample liquid. Then, 3 mL boric acid
 5 absorbing solution (20 g/L) and 50 μ L pH indicator (methyl red: bromocresol green = 1:5, V/V) were added in the inner chamber of Conway dish. In outer chamber, 3 mL potassium carbonate saturated solution and 1 mL sample liquid were added in sequence. The Conway dish was kept sealed and put in 37°C for 2 hrs. The volatile amine gases were absorbed by boric acid absorbing solution. Using 0.1 mol/L hydrochloric acid
 10 (HCl) to titrate, the amount of volatile amines could be calculated by 0.1 mol/L hydrochloric acid usage. The TVB-N value was calculated with the following formula.

$$X = \frac{(v1 - v2) \times C \times 14}{m \times 5/100} \times 100$$

[00110] where, X is TVB-N content in sample (mg/100g), $v1$ is the volume of 0.1 mol/L HCl used for actual test (mL), $v2$ is the volume of 0.1 mol/L HCl used for control test
 15 (mL), C is the HCl concentration (mol/L), 14 is the weight of nitrogen, and m is the weight of sample (g).

[00111] **Example 3D: Sample Preparation and Images Collecting for DCNN Training**

[00112] First, a sensor is attached to the polyvinylchloride (PVC) film. To ensure the
 20 consistency of image background, white 0.45 μ m polytetrafluoroethylene (PTFE) filter membrane was placed beneath the colorimetric barcode-PDMS film as the background. Then, the barcode-PDMS/PTFE was glued on the commercial transparent PVC film with barcode layer facing toward PVC film.

[00113] Next, for meat packaging procedures, procedures were similar to that
 25 commonly used in the supermarket. Briefly, 150 ± 10 g of fresh meat without any pretreatment was put in a plastic box and the PVC film was used for packaging the box. For one package, four sensors were attached on the inner side of PVC film. Then meat was stored at 25°C or 4°C. The schematic diagram and digital image of fully packaged meat were shown in the FIG. 3F.

30 [00114] As for sensor image collection for DCNN training, to collect sensor data, 20 ± 2 photos were taken for each sensor at a certain storage interval without opening the

package. 8 intervals that covered the spoilage process were selected. Noted that the angles and light which are included in the input images were changed for training to fully mimic real testing scenarios.

[00115] Example 3E: Machine Learning

5 **[00116]** During the training and testing, the images were resized to 224 * 224 as input. Through the ResNet 101 backbone and a three dimension Softmax layer, the model predicted the estimated freshness state. At the beginning of training, the learning rate was set to 0.1, and then reduced to 0.05 and 0.01 when the test accuracy exceeded 70% and 80%, respectively. The momentum was set to 0.9 and the weigh decay was set to
10 0.0001. 900 epochs were totally trained.

[00117] Example 4: Discussion of Portable Food Freshness Prediction Using the Present Colorimetric Barcode with Deep Convolutional Neural Networks

[00118] Herein, colorimetric barcode combinatorics was integrated with DCNN to form a system that concurrently provides scent fingerprint and intelligent fingerprint
15 recognition (FIG. 1B). The present system, which may be referred herein as deep colorimetric olfactory-barcoding, is usable for automatically screening the freshness of different types of meats.

[00119] The colorimetric barcode can be made up of, for example, 20 different types of porous nanocomposite formed of dye-loaded chitosan nanoparticles (CNPs-Dye)
20 embedded on cellulose acetate (CA). The halochromic dyes in the barcode cross-react according to the type and concentration of gases to form a scent fingerprint (in the form of a colorful barcode) that is identified using DCNN. Using fully supervised training techniques and 3475 labeled barcode images as the training source, a DCNN that predicted meat freshness with an overall accuracy of 98.5% was obtained. Incorporating
25 DCNN into the smartphone software enables a user to identify the freshness of meats within 30 seconds by simply scanning the barcode. The present system is fast, automated, accurate and non-destructive. As the system uses common biodegradable and biocompatible materials, and was studied under typical food packaging conditions, it is applicable everywhere along the food supply chain for real-time monitoring of food
30 freshness.

[00120] To make the colorimetric strips, the gas sensitive barcodes were patterned on PDMS as a chemically inert, moisture-resistant and transparent substrate. The barcode

was made of porous nanocomposites formed of dye-containing chitosan nanoparticles (CNPs-Dye) embedded in a CA matrix (CNPs-Dye/CA) (FIG. 2A). The CNPs-Dyes were prepared according to the ionic gelation method using sodium tripolyphosphate as the cross-linker. Both chitosan and CA are biodegradable and non-toxic, making them biocompatible and environmentally friendly options for screening food freshness. To realize cross-reactivity between the bars of the barcode in the same way human olfactory receptors cross-react, 20 different CNPs-Dye/CA solutions were created, one for each bar of the barcode. Each CNPs-Dye/CA solution contained a different type of halochromic dye whose chromophore changes color according to the surrounding pH (FIG. 4A). Each of the 20 different dyes responds within a different pH range, covering the detection range of gases emitted from rotting meats. With the spoilage of meat, the concentration of bioamines in the sealed package increases due to microorganism-induced protein degradation and gas accumulation effect. Take bromothymol blue (BPB, the first strip) as an example, the basic bioamines led to a splitting of the hydroxyl group of acid BPB (FIG. 4B), which results in a visible color change from yellow (protonated form) to blue (deprotonated form). When exposed to different gases with varying concentrations, each bar of the barcode displays a different color or range of coloration. This colorful barcode forms the gas fingerprint.

[00121] To deposit the gas sensitive barcodes on PDMS, hydrophilic barcode patterns were created on PDMS with thickness of 2 mm using a shadow mask and oxygen plasma processing (30%, 2 mins) (FIG. 2B). Drop-casting each of the twenty CNPs-Dye/CA solutions onto each successive bar within 10 mins forms the colorimetric barcode. Each bar was 1 mm long and 0.6 mm wide. The gap between the neighboring bar was 1 mm.

[00122] Gas transfer and sensing stability depend on the microstructure of the nanocomposites and their ability to preserve the dye. Scanning electron microscopy imaging showed that while CNPs-Dye themselves formed aggregates (FIG. 2C, bottom row image), they were well distributed on the interweaved fibers that form the porous 3D structure of the CNPs-Dye/CA (FIG. 2C, bottom row image). Such a porous 3D structure provided abundant gas sensing sites. A dye leakage experiment was performed by immersing films of CNPs-Dye and CNPs-Dye/CA in water and characterizing the absorbance of the supernatant. Tetraiodophenolsulfonephthalein (TET) with

characteristic absorbance at 435 nm was used as a model dye. For films of TET-containing chitosan nanoparticles (CNP-TET) and CNP-TET embedded in CA (CNP-TET/CA), the supernatant had negligible absorbance at 435 nm and no visible color (FIG. 2D and 2E). In contrast, the supernatant for a film of TET embedded in CA (CA/TET) was yellow. When immersion time increased from 0 to 84 hrs at room temperature, the peak intensity at 435 nm increased gradually from 0 to 1.51. These results showed that strong intramolecular forces between negatively charged deprotonated hydroxyl groups ($-O^-$) of TET and positively charged protonated amine groups ($-NH_3^+$) of chitosan prevented TET from leaking out of the porous composite (inset in middle image FIG. 2A). Weak Van der Waals forces between CA and TET in the CA/TET film led to large dye leakage. The strong interaction in CNP-TET/CA that avoids dye leakage ensures the safety of barcode in food packaging.

[00123] To test and optimize the sensing performance of the colorimetric barcode, the strip was exposed to methylamine (MA) gas. Increasing concentrations of MA gas turned the barcode on the strip from yellow to deep blue (FIG. 5). The barcode response was quantitatively described by measuring the Euclidean distance (ED) of the RGB values of the barcode before and after exposure to MA. Exposure to higher concentrations of MA increased the ED value, indicating that MA activated the chromophore and the barcode functioned as a scent sensor (FIG. 6). All the CNP-TET/CA barcodes (formed with different mass ratio of CNP-TET:CA) showed a higher response than both CNPs-TET and CA-TET. This enhanced performance was attributed to the 3D porous structure of CNPs-TET/CA, which facilitates gas transfer and provides more sensing sites. Based on the performance of the different barcodes shown in FIG. 2F, the mass ratio of 1:4 was chosen for all subsequent experiments.

[00124] Proteins in rotting meats are microbially decomposed into peptides and amino acids. Over time, these components are further broken down into low-grade amines and ammonia (NH_3), which are useful freshness indicators. To assess whether the present colorimetric barcode strip can be used to monitor meat freshness, the response of the strip to multiple gases typically detected from rotting meats was tested, including ammonia (NH_3), MA, trimethylamine (TMA), putrescine (PUT) and cadaverine (CAD). Like mammalian olfactory receptors where one scent molecule can bind to different receptors and each receptor responds to a range of scents to form a complex scent

fingerprint, one type of gas can trigger several bars of the present barcode and each bar responds to several gases to form a colorful combination of bars that represent the fingerprint for these 5 amine gases. FIG. 7A shows the colorimetric barcode obtained using a scanner (Canon, LBP7010C, 600 dpi) after exposure to different concentrations of individual amine gases (FIG. 7E).

[00125] The data was processed using traditional ED value, PCA, and HCA methods. EDs of R, G, and B values for each bar of the barcode before and after exposure to the amine gases were calculated. The estimated limit of detection (LOD) for NH₃, MA, TMA, PUT and CAD were below 50 ppb at signal to noise ratio of 3 (see Table 1 and formula below).

[00126] Table 1 – LOD for various gases

	NH ₃	MA	TMA	CAD	PUT
LOD-ppb	18.15	17.66	17.80	24.38	21.60

$$S = \sum_{i=0}^{20} (|R_{Analyte} - R_{Control}| + |G_{Analyte} - G_{Control}| + |B_{Analyte} - B_{Control}|)$$

$$SD = \sqrt{\frac{\sum_{i=0}^{20} ((R_{Control} - \bar{R})^2 + (G_{Control} - \bar{G})^2 + (B_{Control} - \bar{B})^2)}{n - 1}}$$

[00127] As mentioned above, the limits of detection (LODs) of NH₃, MA, TMA, CAD and PUT. The LOD is obtained at signal (S) to noise (N) ratio of 3, where S is defined as the difference between blank control (*Control*) and exposed groups (*Analyte*), N refers to the standard deviation (SD) of blank control groups, and n represents eight independent experiments. The blank control groups were performed by exposing the barcode in air without analytes.

[00128] Corresponding to color changes seen in the barcode, higher concentrations of amine gases displayed higher ED values. ED values of the present colorimetric barcode quantitatively describe the concentration of the gases. In FIG. 7B, ED values have overlaps under 20 ppm, and were separate from each other above 20 ppm. Thus, the ED value for different gases before and after exposure to 20 ppm of the gas was investigated. Each gas displayed a distinctive ED value ($n = 8$, $*p < 0.001$), demonstrating that NH₃, MA, TMA, PUT, and CAD have specific gas fingerprints at 20 ppm (FIG. 7C). The

reason is that different chemical environments and the number of amino ($-\text{NH}_2$) groups in these five amine gases induce different color combinations in the barcode.

[00129] PCA and HCA, two widely used classification methods, were further used to evaluate a 50×60 (5 amine gases $\times$ 10 concentration $\times$ 60 RGB values) dimensional matrix acquired from the colorimetric barcode. The gas concentrations examined were
 5 between 20 and 100 ppm. PCA creates several independent PCs that maximally represent the whole matrix. From the PCA score plot, it can be seen that 6 PCs are required to account for 90% of the total variance, 8 PCs for 95%, and 10 PCs for 99% (FIG. 8). The 3D score plot of PCA using the first three PCs (76.9% of the total variance)
 10 shows that clusters of 5 amine gases are clearly separated (FIG. 9).

[00130] With HCA, Ward's minimum variance method was used to determine gas-induced variations of the 60 dimensional RGB colorimetric barcode. The generated cluster-tree showed the 5 amine gases formed clearly separate clusters between 20 ppm and 100 ppm (FIG. 7D). The PCA score plot and HCA cluster tree both intuitively
 15 reveal that the present barcode can effectively discriminate NH_3 , MA, TMA, CAD, and PUT based on a combination of cross-reactive colorimetric sensors. Together, these results demonstrate that it is possible to use the present colorimetric barcode to monitor meat freshness and other microbial metabolic processes in fermented foods such as wine, vinegar and cheese that are known to release CAD and PUT.

20 [00131] As a proof-of-concept, the present colorimetric barcode was used to monitor the freshness of three types of meat—chicken, fish, and beef. To predict meat freshness accurately, DCNN was used to process and recognize the complex multi-dimensional scent fingerprint data. DCNN architecture, which is an end-to-end network with several nonlinear activation functions, is suitable for non-linear multi-dimension data analysis.

25 [00132] The colorimetric barcodes were glued on and faced toward widely used transparent poly(vinyl chloride) (PVC) meat packaging films without touching meat samples (FIG. 3F). Because the barcode is flexible and transparent, it integrates easily with existing polystyrene and PVC packaging materials. The meat packages were stored at 25°C , and images of the barcode were taken at different time intervals using a
 30 smartphone camera without opening the package (FIG. 10). To classify the barcodes into different categories of freshness, the total volatile basic nitrogen (TVB-N) value of the meats was measured using the Conway dish method, an international standard for

determining meat freshness (FIG. 11). Here, in order not to interfere with the barcode image collection, other packages of meat were prepared with the same procedures for TVB-N testing. Meat with TVB-N value ≤ 15 mg/100 g is considered fresh, 15–20 mg/100 g is cooking edible but less fresh, and ≥ 20 mg/100 g indicates inedible and spoiled. Then, these images were classified according to these three standard categories of freshness—fresh, less fresh, and spoiled. As there are variations of TVB-N threshold for meat with different types and sources, a detailed investigation of TVB-N values of a specific meat is suggested to ensure the accurate classification.

[00133] A total of 4161 images of meat and barcode for the three kinds of meat were obtained, forming the dataset for DCNN evaluation of meat freshness. The 4161 images were randomly divided into 3475 images for model training and 686 images for testing (FIG. 3A). A three-class image classification network was designed using the ResNet 101 backbone involving input, multiple convolution (conv.), full connection (FC), and output layers. This trained convolutional neural network extracts features of the barcode from the images and classifies the barcodes into one of the three categories of freshness. As epochs (number of passes the algorithm completes through the entire dataset) increase, the loss that represents the difference between predicted and actual result approaches 0 and the training accuracy improves (FIG. 3B).

[00134] After training, the 686 test images from the dataset were used to evaluate how accurately DCNN classifies the barcode images. When a barcode image is input into the classification network, the freshness category that the meat most likely belongs to is given (denoted by circles of output in FIG. 3A). For the three kinds of meat, the images were classified with 100% accuracy for the spoiled category; in chicken, accuracy was 99.0% for fresh, 96.3% for less fresh; in fish, accuracy was 98.1% for fresh, 98.8% for less fresh; in beef, accuracy was 95.9% for fresh, 98.8% for less fresh (FIG. 3C and Table 2 below).

[00135] Table 2 shows accuracy comparison of DCNN and Euclidean distance for meat freshness evaluation based on colorimetric barcode. DCNN has an overall higher accuracy rate (98.5%) than ED (61.7%).

	Accuracy based on Euclidean distance (%)			Accuracy based on DCNN (%)		
	Fresh	Less fresh	Spoiled	Fresh	Less fresh	Spoiled
Chicken	35.0	55.0	100	99.0	96.3	100
Fish	90.0	30.0	90.0	98.1	98.8	100
Beef	40.0	35.0	100	95.9	98.8	100
Total		61.7			98.5	

[00136] The confusion matrix for the 686 images (FIG. 3D) and subconfusion matrices for chicken, fish and beef (FIG. 12) whose diagonal show the number of correctly classified samples further illustrate the classification accuracy. DCNN classification reached an overall accuracy of 98.5%.

[00137] For comparison, ED values were used to predict meat freshness. To obtain a reference curve, TVB-N values were measured and the ED values of eight samples for each type of meat stored at different time intervals (0 to 48 hrs) were calculated (FIG. 13). Before calculating the ED values, light effects in the images were corrected for using the center of the colorimetric barcode as a reference point for white balance, which is a process of removing unrealistic color casts in images. Both TVB-N values of the samples and ED values of the colorimetric barcodes increased when meat storage intervals increased (FIG. 13). Based on the TVB-N values, the meat samples were categorized as fresh, less fresh, and spoiled. The range of ED values corresponding to each category of freshness was accordingly calculated, which was used for prediction of the freshness of an unknown meat sample.

[00138] Twenty colorimetric barcode images were randomly selected for each freshness category to assess the prediction accuracy of ED. In chicken, ED values achieved prediction accuracy of 35.0% for fresh, 55.0% for less fresh, and 100.0% for spoiled; in fish, it achieved 90.0% for fresh, 30.0% for less fresh, 90.0% for spoiled, and in beef it was 40.0% for fresh, 35.0% for less fresh, 100.0% for spoiled (FIG. 3C). ED analysis showed an overall accuracy rate of 61.7%, which is far lower than DCNN classification (98.5%); fresh and less fresh groups had the poorest accuracy rates. It was also found that ED values between fresh and less fresh groups for chicken, fish, and beef samples were all not significantly different ($p > 0.05$, FIG. 14 to 14C), indicating that it is difficult to distinguish fresh from less fresh using ED values. This low prediction accuracy and discrimination efficiency are because ED values are affected by photo shooting conditions such as angle, zoom and lighting. In contrast, the model

training processes in DCNN exclude most of these effects. Furthermore, because the RGB values of the barcode do not change linearly with the concentration of the target analyte, using the non-linear DCNN method for data processing is more suitable in this case. To automate the overall screening procedure, DCNN was incorporated in a smartphone application (APP), NTUFoodCheck. The APP provides a user-friendly interface for image collection, image uploading, and real-time display of the results (FIG. 3E). Users simply need to capture the colorimetric barcode image within the APP and meat freshness computed by DCNN can be displayed on the screen within 30 seconds. To further validate the applicability of the system in practical refrigeration situations, the present colorimetric barcode was integrated in fish package that were stored at 4°C. 112 images (eight sensors × two photos for each sensor × seven time points) were collected at storage interval from 0 to 5 days and then uploaded in the system for freshness prediction (FIG. 15A and 15B). Based on TVB-N values as the true freshness indicator (FIG. 16), the overall prediction accuracy of 92.0% was obtained. The designed APP can be downloadable onto any smartphone or portable devices, thus the present system can be used by both consumers and food suppliers to screen and/or monitor meat freshness in real-time. The issue regarding barcode image difference caused by camera setting could be solved by embedding domain adaptation in the pipeline of deep learning. In case of no cameras available, human can also roughly distinguish different freshness stage based on reference pictures (FIG. 10).

[00139] Compared with previous food monitoring researches, the present barcode system shows good compatibility with food supply chains (FIG. 17). On the one hand, the present colorimetric barcode with flexibility and editable size could be fully integrated with many packaging materials and techniques. On the other hand, colorimetric barcode recognition can be conducted in a short time (30 seconds) without bulky wiring, which is compatible with fast food supply chains. Moreover, the present system was tested in real application scenarios by using meat packages in commercial PVC film rather than only bioamine sensing in tube. Satisfactory accuracy (98.5%) by using DCNN technique indicates the feasibility of the system integration with food supply chains. Furthermore, by utilizing materials with different gas responsive ability, the present system can recognize other volatile flavor components such as ethylene,

aldehydes, polyphenols and sulfides, which could provide a platform for food quality control or species identification.

[00140] In summary, cross-reactive colorimetric barcodes were combined with DCNN to build a simple platform for rapid, non-destructive monitoring of meat freshness in real-time via a smartphone APP interface. Like the mammalian olfactory system, the cross-reactive colorimetric barcode herein detected gases released by rotting meats and formed a scent fingerprint. DCNN was used to identify this fingerprint, allowing the meats to be rapidly categorized as either fresh, less fresh, or spoiled with an overall prediction accuracy of 98.5%. The accuracy is much higher than the commonly used ED analysis (61.7% overall accuracy). Unlike TVB-N measurements (the current standard for measuring meat freshness), the present technology is non-destructive, automated and enables real-time monitoring. Because the colorimetric barcode can be easily mass-produced and the components are biodegradable and non-toxic, the present system can be widely used in the food industry and by the public. It offers consumers and all stakeholders in the food supply chain a way to monitor and/or screen food freshness and establish public confidence.

[00141] **Example 5: Commercial and Potential Applications**

[00142] Mammal olfactory system is an efficient scent screen system based on the integration of olfactory receptor combinatorics with central nervous system. However, system-level mimicry of olfactory system is still difficult to realize as large data dimension with nonlinearities complicated data statistics, which limit the discrimination accuracy and systematic operability. Herein, a deep colorimetric olfactory-barcoding that integrates cross-reactive sensing strips with DCNN for barcode recognition. As a proof of this concept, a food freshness monitoring system was developed by using 20 kinds of porous chitosan nanoparticles/dyes/cellulose acetate nanocomposites as barcode. With fully supervised training on 3475 labeled barcode images, a DCNN with an overall prediction accuracy of 98.5% was obtained. DCNN was incorporated with software on smartphone which could display food freshness in real-time by scanning the barcode. This deep colorimetric olfactory-barcoding provides an automated platform for accurate, efficient, real-time, non-destructive, facile and high-throughput scent screening with wide application in food safety, public security, and even disease diagnosis.

[00143] While the present disclosure has been particularly shown and described with reference to specific embodiments, it should be understood by those skilled in the art that various changes in form and detail may be made therein without departing from the spirit and scope of the present disclosure as defined by the appended claims. The scope of the present disclosure is thus indicated by the appended claims and all changes which come within the meaning and range of equivalency of the claims are therefore intended to be embraced.

CLAIMS

1. A colorimetric barcode for olfactory detection of one or more scent molecules emitted from a sample, the colorimetric barcode comprising:
 - 5 a polymeric substrate;
 - porous nanocomposites patterned as a barcode on the polymeric substrate,
 - wherein each of the porous nanocomposites is patterned as a bar of the barcode,
 - wherein each of the porous nanocomposites comprises a matrix and a dye-containing carrier embedded in the matrix, and
 - 10 wherein each bar of the barcode comprises one dye-containing carrier which is different from another dye-containing carrier in each of the other bars.
2. The colorimetric barcode of claim 1, wherein the dye-containing carrier comprises a carrier formed from a polymer, wherein the polymer is cross-linked with a
15 vapochromic dye.
3. The colorimetric barcode of claim 2, wherein the polymer has an amine functional group.
- 20 4. The colorimetric barcode of claim 2, wherein the polymer comprises chitosan, acrylamide copolymerized 2-(dimethylamino)ethyl methacrylate, polydiacetylene, or polyaniline.
5. The colorimetric barcode of any one of claims 2 to 4, wherein the vapochromic
25 dye comprises (i) a deprotonated hydroxyl group or (ii) a deprotonated carboxyl group, which interacts with the polymer to have the vapochromic dye confined within the matrix.
6. The colorimetric barcode of any one of claims 2 to 5, wherein the vapochromic
30 dye comprises bromothymol blue, bromocresol green, methyl red, curcumin, alpha-naphtholphthalein, nitrazine yellow, nile blue, bromocresol purple, alizarin red S, alizarin, chlorophenol red, rosolic acid, fluorescein, m-cresol purple, thymol blue,

cresol red, p-naphtholbenzein, brilliant yellow, pyrocatechol violet, and/or tetraiodophenolsulfonephthalein.

7. The colorimetric barcode of any one of claims 2 to 6, further comprising a cross-linker for forming the carrier from the polymer, wherein the cross-linker comprises tripolyphosphate or glutaraldehyde.
8. The colorimetric barcode of any one of claims 1 to 7, wherein the matrix comprises a cellulose-based material.
9. The colorimetric barcode of any one of claims 1 to 8, wherein the polymeric substrate is moisture-resistant and transparent.
10. The colorimetric barcode of any one of claims 1 to 9, wherein the polymeric substrate comprises poly(dimethylsiloxane), polyvinyl pyrrolidone, thermoplastic polyurethane, a polyolefin elastomer, or polyethylene terephthalate.
11. A method of forming the colorimetric barcode of any one of claims 1 to 10, the method comprising:
 - (i) providing a dye-containing carrier;
 - (ii) mixing the dye-containing carrier with a matrix solution to form a porous nanocomposite, wherein the porous nanocomposite comprises a matrix having the dye-containing carrier embedded in the matrix;
 - (iii) repeating steps (i) and (ii) to form porous nanocomposites, wherein each of the porous nanocomposites comprises one dye-containing carrier which is different from another dye-containing carrier in each of the other porous nanocomposites; and
 - (iv) depositing the porous nanocomposites on a polymeric substrate to form bars of the colorimetric barcode.
12. The method of claim 11, wherein providing the dye-containing carrier comprises mixing a vapochromic dye with a polymer.

13. The method of claim 12, wherein providing the dye-containing carrier further comprises mixing the vapochromic dye with the polymer in the presence of a cross-linker.

5 14. The method of any one of claims 11 to 13, wherein depositing the porous nanocomposites on the polymeric substrate comprises:

forming trenches in the polymeric substrate; and

depositing the porous nanocomposites into the trenches to form the bars of the colorimetric barcode,

10 wherein each bar of the barcode comprises one dye-containing carrier which is different from another dye-containing carrier in each of the other bars.

15 15. The method of claim 14, wherein depositing the porous nanocomposites on the polymeric substrate further comprises rendering the polymeric substrate hydrophilic by exposing the trenches to oxygen plasma treatment.

16. A method of identifying freshness of food using the colorimetric barcode of any one of claims 1 to 10, the method comprising:

20 providing a training dataset comprising training image data comprising training pairs of the colorimetric barcode of any one of claims 1 to 10 and a corresponding freshness of the food; and

training a neural network with the training dataset to obtain the trained neural network.

25 17. The method of claim 16, further comprising:

identifying the freshness of the food using the trained neural network.

18. The method of claim 16 or 17, wherein the trained neural network is a deep convolutional neural network (DCNN).

19. A non-transitory computer-readable medium storing computer executable code comprising instructions for identifying freshness of food using the colorimetric barcode of any one of claims 1 to 10.
- 5 20. A computer executable code comprising instructions for identifying freshness of food using the colorimetric barcode of any one of claims 1 to 10.