(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
12 May 2022 (12.05.2022)

WIPO | PCT

(10) International Publication Number
WO 2022/099082 A1

(51) International Patent Classification:
A61B 5/00 (2006.01) *A61B 5/11* (2006.01)
A61B 5/0205 (2006.01)

(21) International Application Number:
PCT/US2021/058346

(22) International Filing Date:
05 November 2021 (05.11.2021)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/110,237 05 November 2020 (05.11.2020) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO,

(54) Title: FIBER-OPTIC INTEGRATED TEXTILES WITH EMBEDDED FREEZE-DRIED CELL-FREE REACTIONS FOR WEARABLE SENSORS

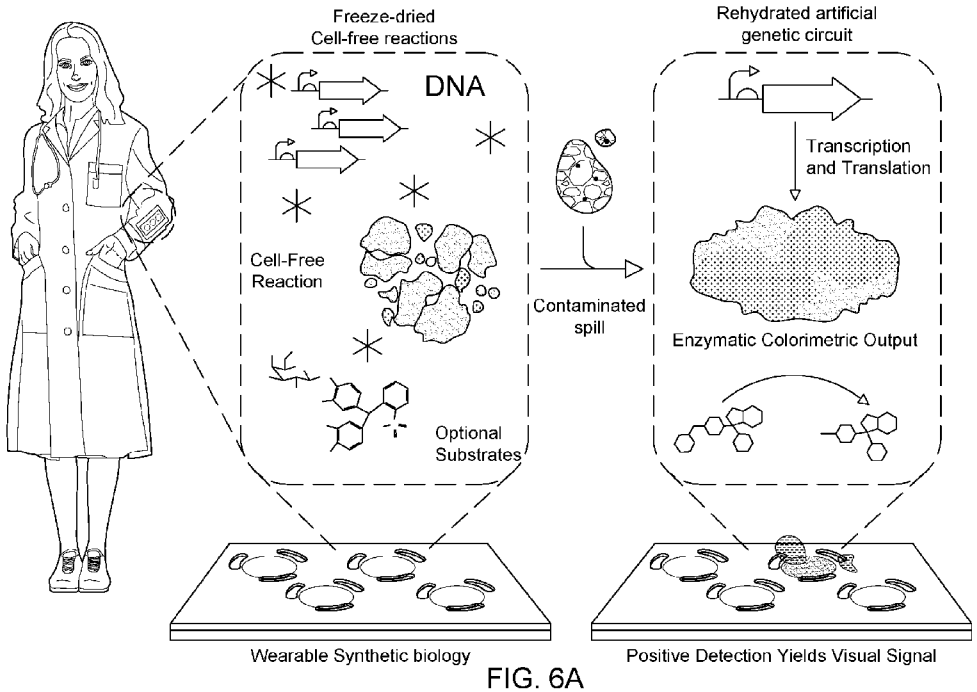


FIG. 6A

(57) Abstract: Embodiments of various aspects described herein are directed to an aqueous solution-activated sensor fabric. The fabric includes an excitation plastic optical fiber (POF) and an emission POF combined with a porous hydrophilic material into a flat web structure. The fabric also includes a freeze-dried cell free (FDCF) synthetic biological compound in at least a portion of the web structure.

WO 2022/099082 A1

[Continued on next page]

DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, IT, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

FIBER-OPTIC INTEGRATED TEXTILES WITH EMBEDDED FREEZE-DRIED CELL-FREE REACTIONS FOR WEARABLE SENSORS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of and priority to U.S. Provisional Patent Application No. 63/110,237, filed November 5, 2020, which is hereby incorporated by reference herein in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

[0002] This invention was made with government support under HDTRA1-14-1-0006 awarded by the Department of Defense/Defense Threat Reduction Agency. The government has certain rights in the invention.

TECHNICAL FIELD

[0003] The present invention relates generally to wearable sensors. More specifically, the present invention relates to a wearable sensing platform based on cell-free synthetic biology reactions added to flexible substrates and textiles.

BACKGROUND

[0004] Synthetic biology has provided control of biological systems and has led to developments in biotechnology and medicine. Modular biosensors, genetic logic gates, and output effectors are a part of customized biological circuits. In parallel, recent developments in wireless technology, wearable electronics, smart materials, and functional fibers including mechanical, electrical and optical properties have included the development of biosensing systems. Even though genetically-encoded sensors have been incorporated into bench-top diagnostics, examples of wearable devices using these tools are limited. Only a few demonstrations of hygroscopically actuated vents and response to induction molecules have been achieved using living engineered bacteria encapsulated in flexible substrates and hydrogels in a wearable format.

[0005] The combination of living engineered bacteria with flexible substrates and hydrogels in a wearable format encounters several limitations, particularly sustaining living organisms within wearable devices for extended periods. In practice, retaining viability and function of wearable sensing systems based on living cells requires nutrient delivery, waste extraction, as well as temperature and gas regulation, all of which involve numerous technological hurdles.

Genetically engineered cells can also pose biocontainment or biohazard concerns, particularly if integrated into consumer-level garments, leading to stringent regulatory pathways in many critical applications. Moreover, continually evolving cell populations suffer mutational pressures over time, resulting in potential loss of the genetic phenotype and function.

[0006] Thus, there is a need for a new approach in synthetic biology to resolve the mismatch between practical requirements of wearable use and operational limitations of available biomolecular circuits for sensing and response. The present disclosure is directed to solving these and other problems.

SUMMARY

[0007] According to one implementation, an aqueous solution-activated sensor fabric includes an excitation plastic optical fiber (POF) and an emission POF combined with a porous hydrophilic material into a flat web structure. The fabric also includes a freeze-dried cell free (FDCF) synthetic biological compound in at least a portion of the web structure.

[0008] According to another implementation, an aqueous solution-activated sensor includes a chamber formed in a flexible material and synthetic biological components disposed in the chamber. A first UV-Vis light-transmitting medium provides a first optical connection from the interior of the chamber to an exterior of the chamber. A port fluidly connects an exterior surface of the flexible material to an interior of the chamber. The port allows an aqueous solution in contact with the exterior surface to be wicked to the interior. The FDCF synthetic biological components are hydrated upon exposure to the aqueous solution to form rehydrated synthetic biological components. The rehydrated synthetic biological components are formulated to provide an optical signal transmittable through the light transmitting medium. The signal is responsive to the presence or absence of a triggering compound in the aqueous solution wicked to the interior of the chamber.

[0009] According to another implementation, a method for making an aqueous solution-activated sensor includes providing a layer of a first material, providing a layer of a second material on the top surface of the first material, and providing a layer of a third material on a top surface of the second material. A portion of a top surface of the first material defines a bottom wall of a chamber. The second material also includes a first continuous open space on the portion of the top surface that defines the bottom wall of the chamber. The second material defines a side wall of the chamber. The third material also includes a second continuous open space disposed above the chamber. The third material defines a top wall of the chamber including a port defined

by the second continuous open space. The method further includes adding synthetic biological components into the chamber, and freeze drying the synthetic biological components.

[0010] The above summary is not intended to represent each implementation or every aspect of the present disclosure. Additional features and benefits of the present disclosure are apparent from the detailed description and figures set forth below.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] The disclosure will be better understood from the following description of exemplary embodiments together with reference to the accompanying drawings.

[0012] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0013] FIGs. 1A-1C illustrate an aqueous solution-activated sensor fabric, according to some implementations of the description. FIG. 1A depicts a top schematic, FIG. 1B depicts an isometric view, and FIG. 1C depicts a detailed view.

[0014] FIG. 2 illustrates an implementation of a fabric based detector, according to some implementations of the description.

[0015] FIGs. 3A-3C depict an implementation of a fabric based detector including a hydrophobic material, according to some implementations of the description. FIG. 3A is a first view and FIG. 3C is a second view, both illustrating the hydrophobic material on a web structure. FIG. 3C depicts a close up schematic view of the hydrophobic material.

[0016] FIGs. 4A-4B depict an implementation of an aqueous solution-activated sensor, according to some implementations of the description.

[0017] FIGs. 5A-5B illustrate another implementation of an aqueous solution-activated sensor, according to some implementations of the description. FIG. 5A is an exploded layer view and FIG. 5B is a front cross-sectional view through one of the chambers.

[0018] FIGs. 6A-6H illustrate wearable cell-free synthetic biology, according to some implementations of the description. FIG. 6A depicts how freeze-dried cell-free reactions can be embedded in reaction sachets or chambers that are distributed throughout garments for use by soldiers, clinicians, and first responders. FIG. 6B depicts a schematic of the layer-by-layer assembly of the wearable devices. FIG. 6C depicts an array of assembled reaction chambers showing the elasticity (center) and flexibility (right) of the devices. FIG. 6D depicts portals cut into the outermost layer. FIG. 6E-6F, depict various types of synthetic biology circuits can be freeze-dried in these wearable devices, including; constitutively expressed outputs (FIG. 6E),

transcription factor-regulated circuits for small molecule detection (FIG. 6F), toehold switches for nucleic acid-sensing (FIG. 6G), and riboswitches to detect various small molecules (FIG. 6H).

[0019] FIGs. 7A-7C depict assembly layers and sample activation of colorimetric wFDCF reactions with constitutive P_{T7}::LacZ module, according to some implementations of the description. FIG. 7B depict the activation of colorimetric wells or reaction chambers with a control rehydration solution. FIG. 7C depict the activation of the reaction chambers with a rehydrating solution including a triggering compound.

[0020] FIGs. 8A-8C depict sample activation of wFDCF colorimetric devices and a bracelet, according to some implementations of the description. FIG. 8A depicts activation of colorimetric Ebola virus DNA toehold wFDCF sensor. FIG. 8B depicts port wicking into reaction chambers containing reaction disks using dd-H_2O fluid splash. FIG. 8C depicts activation of the wearable colorimetric bracelet with four independent Ebola virus DNA toehold sensors.

[0021] FIGs. 9A-9G depict the design and validation of fluorescent and luminescent freeze-dried cell-free synthetic biology wearables, according to some implementations of the description. FIG. 9A details of assembly and activation of fiber-optic based wFDCF module for fluorescence/luminescence output. FIG. 9B top - diagram depicting the layers of the assembled device; bottom - cross-sectional view of the interior of the device. FIG. 9C depicts comparison of fluorescent signal after rehydration of wFDCF constitutive sfGFP template as compared to control. FIG. 9D depicts activation of FDCF riboswitch in a wearable device as compared to a control. FIG. 9E depicts a demonstration of fluorescent aptamer being activated by a substrate as compared to a control. FIG. 9F illustrates luminescence output detected from an HIV toehold sensor with nanoLuciferase operon. FIG. 9G illustrates wearable detection of organophosphate nerve agents.

[0022] FIGs. 10A-10D illustrate that concentrating PURE cell-free reactions increases reaction kinetics, according to some implementations of the description. FIG. 10A is a schematic of reaction concentration through the lyophilization of PURExpress® (New England Biolabs, Inc., Ipswich, MA) reactions at varying volumes followed by rehydration at a set volume. FIG. 10B depicts representative images of PURE reactions with a LacZ output over one hour, at various concentrations. FIG. 10C depicts quantified PURExpress® reactions with a LacZ output. FIG. 10D depicts the half-maximal values from the curve fitting the data shown in FIG. 10D.

[0023] FIG. 11 depicts Zika DNA Toehold sensor activation in single mercerized cotton thread, according to some implementations of the description.

[0024] FIG. 12 depicts antibiotic resistance sensors for *spa*, *ermA* and *mecA* genes using in-wearable sensor demonstrate specific orthogonality, according to some implementations of the description.

[0025] FIG. 13 depicts POF fabric compatibility with lyophilized transcription-only fluorescent aptamer reactions, according to some implementations of the description.

[0026] FIG. 14 depicts sensor multiplexing using different fluorescent proteins in a single device, according to some implementations of the description.

[0027] FIGs. 15A-15B depict NanoLuciferase (nLuc) luminescence experiments, according to some implementations of the description. FIG. 15A depicts the dynamic response of a wFDCF Lyme disease RNA toehold switch sensor with luminescence output. FIG. 15B depicts the dynamic response of a wFDCF HIV RNA toehold switch sensor with luminescence output in comparison to constitutive P_{T7}::nLuc expression as a positive control.

[0028] FIGs.16A-16D depict fabrication of polymeric optic fiber (POF) fabric for wFDCF, according to some implementations of the description. FIG. 16A depicts how hydrophilic yarns were weaved along the weft in combination with POFs as warp. FIG. 16B depicts a three-fiber multi-strip design. FIG. 16C depicts a roll of the hydrophilic POF fabric after weaving. FIG. 16D depicts a cut section of the hydrophilic POF fabric with indications in reaction zone and bundle ends.

[0029] FIGs.17A-17G depict fabrication of textile-based wFDCF sensor patch, according to some implementations of the description. FIG. 17A depicts a cut strip of hydrophilic POF fabric that was laser-etched. FIG. 17B depicts examples of prepared wFDCF fabric-elastomer layers and final assembly into a three-well sensor for garment integration. FIG. 17C depicts a schematic of a POF-fabric-elastomer strip for sensing in a single textile layer including two excitation fibers on the sides of an emission fiber. FIG. 17D depicts a schematic of a double POF-fabric-elastomer strip for sensing with dedicated excitation and emission layers. FIG. 17E depicts a schematic of a single excitation or emission POF-fabric-elastomer layer overlaid on an applied elastomer pattern for creating the impermeable reaction wells or chambers. FIG. 17F depicts a finalized three-well sensor wFDCF device with heat shrunk POF covers and Luer connectors for interface with a portable spectrometer device. FIG. 17G depicts a top and bottom views of a final three-well sensor wFDCF device.

[0030] FIGs. 18A-18B depict textile substrate compatibility testing using synthetic biology reactions and sample colorimetric reaction, according to some implementations of the

description. FIG. 18A depicts samples of eight fabric types selected as part of the textile screening for wFDCF compatibility. FIG. 18B depicts a sample wFDCF colorimetric activation in a cellulose matrix square containing a protein synthesis solution.

[0031] FIGs. 19A-19B depict textile screening using model constitutive P_{T7}::LacZ assay, according to some implementations of the description. FIG. 19A depicts a sample well plate containing BSA blocked and unblocked discs of different textile types after constitutive P_{T7}::LacZ expression following a 12-hour run for reactions containing an protein synthesis solution with plasmid or without plasmid as controls. FIG. 19B depicts examples of qualitative traces of colorimetric signals for these different fabric disks using a plate spectrophotometer.

[0032] FIG. 20 depicts a compilation of normalized functional scoring for colorimetric wF-DCF textile screening, according to some implementations of the description.

[0033] FIGs. 21A-21F depict fabrication of wearable microcontroller system with LED illumination and spectrometric capabilities, according to some implementations of the description. FIG. 21A is an exploded isometric view of wearable POF spectrometer components with case and electronics. FIG. 21B is a photograph of an open assembled device. FIG. 21C is a photograph of a fully assembled device ready for imaging. FIG. 21D depict details of a camera used in the device. FIG. 21E is a top view of an assembled device to provide detail of compact electronics arrangement. FIG. 21F depicts the arrangement of a wearable POF spectrometer with wireless connectivity in-garment for wFDCF reaction testing.

[0034] FIGs. 22A-22C depict custom mobile application software, according to some implementations of the description. FIG. 22A depicts a main window of the developed wFDCF sensor mobile application where spectrographic measurements are continuously recorded. FIG. 22B depicts an environmental window of the mobile application displaying geolocation information as well as environmental information. FIG. 22C depicts an excitation window of the application.

[0035] FIGs. 23A-23J depict validation of CRISPR-based FDCF wearable sensors, according to some implementations of the description. FIG. 23A depicts the sensing mechanism of CRISPR-Cas12a system. FIG. 23B depicts a wFDCF mecA CRISPR-based sensor exposed to sample containing mecA trigger. FIG. 23C depicts wFDCF spa CRISPR-based sensor exposed to spa trigger. FIG. 23D depicts wFDCF ermA CRISPR-based sensor exposed to ermA trigger. FIG. 23E depicts experimental detection of mecA CRISPR-based sensor was statistically distinguishable. FIG. 23F is an orthogonality demonstration of mecA / spa / ermA CRISPR-based multi-sensor wearable. FIG. 23G is a plot depicting the orthogonality. FIG. 23H depicts POF end on light up demonstrating the orthogonality. FIG. 23I depicts garment-level integration

of fabric-based wearable synthetic biology sensors. FIG. 23J depicts connection of fabric-based module to wearable POF spectrometer with wireless connectivity capabilities.

[0036] FIG. 24 depicts the limit of detection of wFDCF CRISPR-Cas12a based sensor activated in-fabric, according to some implementations of the description.

[0037] FIG. 25 depicts comparison of Cas13a-based SHERLOCK MRSA RNA-sensing in wFDCF in-fabric prototype against signal in a standardized plate reader, according to some implementations of the description.

[0038] FIGs. 26A-26E depict integrated wFDCF sample Lysis, according to some implementations of the description. FIG. 26A depicts detergent combinations for cellular lysis were tested against CRISPR-Cas12a SHERLOCK reactions. FIG. 26B depicts assembly of the wFDCF with lysis. FIG. 26C depicts in-wearable wFDCF mecA sensors containing a lyophilized lysis buffer challenged with intact *E. coli* cells either containing the target *mecA* gene or a negative control plasmid. FIG. 26D depicts some non-ionic surfactants used as freeze-dried lysis reagents: top row left to right Triton X-100, NP-40, and Tween-20; bottom row left to right Brij-58, Brij-C10, and Brij-S20. FIG. 26E depicts some ionic surfactants used as freeze-dried lysis reagents: left to right; sodium dodecyl sulfate, CHAPS hydrate, and sodium deoxycholate.

[0039] FIGs. 27A-27D depict bioinspired sample-wicking for textile-based wFDCF synthetic biology devices, according to some implementations of the description. FIG. 27A is a schematic of the base cover presented for the textile-based wFDCF synthetic biology devices, as well as the underlying biomechanical mechanism of water collection. FIG. 27B depicts a modified cover for the textile-based wFDCF synthetic biology devices with wicking ports. FIG. 27C depicts a five-second time-lapse of the fluid pinning and port wicking exhibited by the device. FIG. 27D is a photograph of an assembled textile-based wFDCF synthetic biology device including the bioinspired port.

[0040] While the present disclosure is susceptible to various modifications and alternative forms, specific implementations and embodiments thereof have been shown by way of example in the drawings and will herein be described in detail. It should be understood, however, that it is not intended to limit the present disclosure to the particular forms disclosed, but on the contrary, the present disclosure is to cover all modifications, equivalents, and alternatives falling within the spirit and scope of the present disclosure as defined by the appended claims.

DETAILED DESCRIPTION OF THE INVENTION

[0041] The present disclosure can be embodied in many different forms. Representative embodiments are shown in the drawings, and will herein be described in detail. The present disclosure is an example or illustration of the principles of the present invention, and is not

intended to limit the broad aspects of the disclosure to the embodiments illustrated. To that extent, elements, and limitations that are disclosed, for example, in the Abstract, Summary, and Detailed Description sections, but not explicitly set forth in the claims, should not be incorporated into the claims, singly or collectively, by implication, inference, or otherwise. For purposes of the present detailed description, unless specifically disclaimed, the singular includes the plural and vice versa; and the word "including" means "including without limitation." Moreover, words of approximation, such as "about," "almost," "substantially," "approximately," and the like, can be used herein to mean "at," "near," or "nearly at," or "within 3-5% of," or "within acceptable manufacturing tolerances," or any logical combination thereof, for example.

[0042] Embodiments of various aspects described herein are, at least in part, based on the discovery that synthetic biological reactions can be incorporated into wearable devices and fabrics. The synthetic biological reactions can be selected to function as sensors and expand and complement the scope of use available with live biological sensor systems. The various embodiments enable many applications for synthetic biology, allowing utilization in a wide range of wearable substrates (e.g., functional fibers or fabrics) to assess molecular targets difficult to detect through other technologies. The sensors can be used, for example, by first responders, military personnel, and clinicians at risk to exposure to biological pathogens, viruses and chemical toxins.

[0043] Cell-free synthetic biology reactions are self-contained abiotic chemical systems with all the biomolecular components required for efficient transcription and translation. Such systems can be freeze-dried into shelf-stable formats using porous substrates, which allow for robust distribution, storage and use without specialized environmental or biocontainment requirements. Genetically engineered circuits, encoded in DNA or RNA, can be added to freeze-dried, cell-free (FDCF) reactions for activation by simple rehydration. According to some implementations of the disclosure, FDCF genetic circuits are combined with flexible and textile substrates. These can be incorporated and used for the design of practical wearable biosensors. According to some aspects, various wearable freeze-dried, cell-free synthetic biology (wFDCF) sensors for small molecule, nucleic acid, and toxin detection have been made. These sensors can be integrated into flexible multi-material substrates (e.g., silicone elastomers and textiles) using genetically engineered components, including toehold switches, transcriptional factors, riboswitches, fluorescent aptamers, and CRISPR-Cas (e.g., Cas 12a, 13a) complexes.

[0044] FIGs. 1A-1C illustrate an aqueous solution-activated sensor fabric (100), according to some implementations. FIG. 1A depicts a top schematic, FIG. 1B depicts an isometric view, and FIG. 1C depicts a detailed view. The fabric includes an excitation plastic optical fiber (POF)

102, and an emission POF 104 combined with a porous hydrophilic material into a flat web structure 106. The fabric also includes a FDCF synthetic biological component 108 in at least a portion of the web structure. Optionally, the web structure 100 is a woven structure where the excitation POF 102 and emission POF 104 are woven in the warp direction 112, and the hydrophilic material 110 is woven in the weft direction 114.

[0045] The excitation POF 102, and the emission POF 104 include an outer cladding 116. In some implementations, as shown in FIG. 1C, the excitation POF 102 and the emission POF 104 are etched to remove a portion 118 of outer cladding 116. This provides a pathway for light to enter into, or exit out of, the POF along its length.

[0046] FIG. 2 illustrates an implementation of a web structure 200. The web structure 200 is a woven structure including a first layer 202 where the excitation POF 102 is woven in a warp direction 112, and the porous hydrophilic material 110 is woven in the weft direction 114. In some implementations, the excitation POF includes a plurality of substantially parallel POFs. The web structure 200 can optionally include a second layer 204 wherein the emission POF 104 is woven in the warp direction 112, and the porous hydrophilic material is woven in the weft direction 114. In some implementations, the excitation POF 102 includes a plurality of substantially parallel excitation POFs 102. In some implementations, the emission POF 104 includes a plurality of parallel emission POFs 104.

[0047] In some implementations, the FDCF synthetic biological component is spatially contained by being surrounded by patterns of a hydrophobic material. FIG. 3A -3C illustrate one possible configuration. A hydrophobic material 302 is shown in FIG 3A, and shown in outline in FIG. 3B. The synthetic biological component 108 is surrounded by the hydrophobic material 302. The synthetic biological components 108 is absorbed on and in the porous hydrophilic material 110. A port 306 (e.g., a small opening) is included. The port 306 exposes the synthetic biological component 108 to the environment outside of the web structure 106 so that an aqueous solution can enter and make contact with the synthetic biological component 108.

[0048] FIG. 3C depicts a depicts a close up schematic view of the hydrophobic material 302. The hydrophobic material 302 forms a chamber 308, shown as a dashed outline. The port 306 provides a fluid connection to the chamber 308 through a conduit 310. The FDCF synthetic biological components 108 are disposed (e.g., deposited or placed) in the chamber 306 (not shown for clarity). An excitation POF 102 and emission POF 104 are shown passing through the hydrophobic material 302, and through the chamber 308. Additional fibers of POFs can be included. For clarity, the porous hydrophilic material 110 is also not shown. In some implementations, a first end 322 of the excitation POF 102 is treated with a reflective coating. A

second end 332 of the excitation POF 102 can be connected to an excitation source, such as an LED light. In some implementations, a first end 324 of the emission POF 104 is treated with a reflective coating. A second end 334 of the emission POF 104 can be connected to a detector.

[0049] In some implementations, the chamber volume is between about 0.1 μL and about 500 μL (e.g., between about 1 and 150 μL). In some implementations, the port 306 is between 0.1μm^2 and 50 mm^2 (e.g., between 1 μm^2 and 10 mm^2).

[0050] Although illustrated in FIG. 3A-3C as web structure 106, other web structures, such as the web structure 200 (FIG. 2) can also be used. In some implementations, no POFs are used and a top portion 312 of the hydrophobic material 302, all through the hydrophobic material 302 to the chamber 308 (FIG. 3C), is transparent.

[0051] When an aqueous solution contacts the synthetic biological components 108, they are re-hydrated. These can include the various FDCF biological components described herein. If a trigger compound is present in the aqueous solution, a signal output can be observed.

[0052] FIG. 4A and 4B depict an implementation of an aqueous solution-activated sensor 400. FIG. 4A is an exploded perspective layer view and FIG. 4B depicts separated layer of the sensor 400 from a top view. A chamber 402 is formed by a bottom layer 404 of a flexible material, a middle layer 406 of a second flexible material, and a top layer 408 of a third flexible material. The first, second and third flexible materials can have the same or different compositions. The bottom layer 404 defines a bottom wall 414 of the chamber, the area of which is shown in encircled by a dashed line (e.g., the boundary) in FIG. 4B. The boundary defining the bottom wall 414 is provided by a cut out in the middle layer 406. The middle layer 406 defines a side wall 416 of the chamber, by the continuous open space or cut out in the middle layer 406. A top layer 408 defines a top wall of the chamber 418, shown by a dashed outline (opposite an exterior surface 412). Synthetic biological components 108 are disposed in the chamber. A port 410 fluidly connects the exterior surface 412 of the third layer 408 of the flexible material to an interior of the chamber 402. The port is defined by a continuous open space or cut out in the top layer 408. The port 410 allows an aqueous solution in contact with the exterior surface 412 to be wicked to the interior of the chamber 402. The FDCF synthetic biological components are hydrated upon exposure to the aqueous solution to form rehydrated synthetic biological components. The rehydrated synthetic biological components are formulated to provide an optical signal transmittable through a light transmitting medium. The optical signal is responsive to the presence or absence of a triggering compound in the aqueous solution wicked to the interior of the chamber.

[0053] In some implementations, the top layer 404, or a portion thereof, is a UV-Vis light transmitting medium and provides an optical connection to the interior of the chamber 402. In some implementations, at least of portion of flexible material is opaque to UV-Vis light. In some implementations one or more of the bottom layer 404, the middle layer 406 and top layer 408 include an elastomeric material.

[0054] According to some aspects, a dried lysate is disposed in the chamber. In some implementations, the dried lysate is disposed in the chamber between the port 410 and the FDCF biological components 108. Optionally, the dried lysate is absorbed on or in a porous hydrophilic material. In some implementations, a dissolvable membrane or dissolvable material is disposed between the dried lysate and the FDCF biological components 108. The dissolvable material can provide a time delay allowing the lysate to act on components, such as cells and viruses, in the aqueous solution. The aqueous solution, and lysates in the aqueous solution, subsequently contact the biological components 108. In some implementations a delay is provided by a tortuous path. For example, a barrier is provided that is made of material that is impermeable to the aqueous solution but has a tortuous channel. The tortuous channel fluidly connects the dried lysate and the FDCF biological compounds. As used herein, tortuous can include a winding path for the channel creating a large distance for the aqueous solution to flow through, and can include constrictions and narrowing restricting. This geometry delays the flow of the aqueous solution through the tortuous channel.

[0055] In some implementations, a porous hydrophilic material is disposed in the chamber 402. In some implementations, the porous hydrophilic material is treated with a blocking agent. The FDCF biological components 108 can be absorbed in or on the hydrophilic material.

[0056] FIG. 5A and 5B illustrate another implementation of an aqueous solution-activated sensor 500. FIG. 5A is an exploded perspective view and FIG. 5B is a front cross-sectional view through one of the chambers. The cross-section is perpendicular to the direction of the parallel POFs 102, 104. Some aspects are similar to the implementation 400 (FIG. 4A, 4B). For example, sensor 500 features a chamber 402 formed in a flexible material by a bottom layer 404 of the flexible material, a middle layer 406a and 406b of the flexible material (a single middle layer 406 is used in the embodiment shown in FIG. 4A), and a top layer 408 of the flexible material. In this implementations, a first UV-Vis light transmitting medium is the emission POF 104, whereas the first UV-Vis light transmitting medium in FIG. 4A is a portion of the top layer 408. In the implementation, a second UV-Vis light transmitting medium is the excitation POF 102. A port 410 fluidly connects the exterior surface of the third layer 408 of the flexible material to an interior of the chamber 402, similar to the implementation shown in FIG. 4A.

[0057] In some implementations, a portion of an outer cladding of the emission POF 104 is removed or etched, as previously described with reference to FIG. 1C. This provides the first optical connection from the interior of the chamber 402. A portion of an outer cladding of the excitation POF 102 can also be removed or etched to provide the second optical connection to the interior of the chamber 402. One end of the emission POF 104 and excitation POF 102 can be connected to a spectrophotometer. For example, in some implementations, the excitation POF 102 is connected to a light source such as an LED light, and in some implementations the emission POF 104 is connected to a detector, such as a CCD detector. The other end of the emission POF 104 and excitation POF 102 can be treated with a reflective compound to provide a reflective surface.

[0058] In some implementations, light from the reactions enter the POFs through ends that are cut (i.e., transmission through the end of the fiber). Different ways generated light can be absorbed into the POF includes: (a) through the side of the fiber where the cladding has been removed, (b) through the end of the fiber, and/or (c) through some light-focusing material (e.g., some kind of geometric waveguide that can absorb emited photons and route them to the POF).

[0059] In some implementations, an opaque barrier 504 is inserted between the port 410 and both of the emission POF 104 and excitation POF 104. The opaque barrier is selected to reduce or eliminate light transmission from the port 410 to the emission POF and excitation POF. The optical barrier includes fluid connectivity to the chamber 402, for example shown as a gap 505 in FIG. 5B. Any form of fluid connectivity such as holes and perforations through the optical barrier 504 can be use provided light is eliminated or reduced. For example, in some implementations the light is reduced by at least 80%, at least 90%, at least 95%, or at least 99%, when the optical barrier 504 is used.

[0060] Still referring to the implementation depicted by FIG. 5A-5B, the emission POF 104 and the excitation POF 102 are combined with a porous hydrophilic material. For example, the POFs 102, 104 can be interwoven with the porous hydrophilic material providing a woven fabric 110 as previously described and shown in FIG. 1A-1C and FIG. 2. In some implementations, the emission POF 104 is interwoven with a first portion of porous hydrophilic material providing a first woven fabric (e.g., layer 204 in FIG. 2), and the excitation POF is interwoven with a second portion of the porous hydrophilic material providing a second woven fabric (e.g., layer 202 FIG. 2). FIG. 5B only shows a single hydrophobic material 110 for clarity, but multiple layers of hydrophobic material and POFs is also contemplated as a possible implementation.

[0061] In some implementations, at least a portion of the porous hydrophilic material 110 is embedded in the flexible material. For example, a portion of material 110 indicated as region

506, protrudes out of the layers 406a and 406b. The porous hydrophilic material 110 passes from the chamber 402, through region 405 of layer 406a, 406b, and out of the sensor 500 to region 506.

[0062] In some implementations, the sensor 500 includes a plurality of conical spikes 508 perpendicular to the exterior surface and proximate to the port. The conical spikes aid in collecting and attracting aqueous solutions close to the port 410.

[0063] Some implementations relate to methods for making an aqueous solution-activated sensor. The method includes providing a layer of a first material. For example, the layer of the first material can include the bottom layer 404, as depicted in FIG 4A and 4B, 5A and 5B. A layer of a second material is provided on the top surface of the first material. For example, the layer of the second material can include the middle layer 406, 406a or 406b. A layer of a third material is provided on a top surface of the second material. For example, the layer of the third material can include the top layer 408. The method further includes adding synthetic biological components into the chamber.

[0064] In some implementations, the synthetic biological components are freeze dried after being placed in the chamber 402. In some other implementations, the synthetic biological components are freeze-dried or otherwise dried prior to placement in the chamber 402. In some implementations, the FDCF biological components are absorbed on a porous hydrophilic material. The material can be inserted into the chamber 402 through the port 402, for example, where the top layer 408 is made of an elastomeric material.

[0065] In some implementations, the method includes addition of lysate, optionally absorbed on a porous hydrophilic material. Optionally, a time delay barrier, such as a dissolvable barrier or a barrier having a tortuous channel there through, is placed between the lysate and the biological components.

[0066] In some implementations, the method includes curing any one or more of the first material, the second material, and the third material prior to, during, or after providing the first material, second material, or third material as a layer. For example, any one of the materials can comprise a cross linking polymer that cross-links upon heat curing, exposure to oxygen or after adding an initiator or catalyst.

[0067] In some implementations, the method includes solidifying any one or more of the first material, the second material, and the third material from a molten state prior to, during, or after providing the first material, second material, or third material as a layer. For example, the material can be a thermoplastic which is heated, cast to form one or more layers 404, 405, 406a, 406b, or 408 and then cooled so that it solidifies. In some implementations, the thermoplastic is

formed by additive manufacturing such as 3D printed to form the layers. In some implementations the thermoplastic is formed by a subtractive process, such as milling (e.g., CNC machining). In some implementations, one or more of the layers are formed by injection molding.

[0068] In some implementations, the method includes forming, by a polymerization reaction, any one or more of the first material, the second material, and the third material from monomeric precursors, during, or after providing the first material, second material, or third material as a layer.

[0069] ***Hydrophobic materials***

[0070] According to some implementations, any hydrophobic material can be used. For example, a low molecular polymer or oligomer such as a wax. In some implementations, the hydrophobic material is an elastomeric material such as one or more of ethylene propylene diene monomer (EPDM) rubber, a silicone, a neoprene rubber, a natural rubber, a nitrile rubber, a butyl rubber, a thermoplastic elastomer, or any hydrophobic elastomer. In some implementations, the elastomeric material is a silicone.

[0071] ***Porous hydrophilic materials***

[0072] Porous hydrophilic materials can include any material that can be wet by an aqueous solution and adsorbs between 10 wt.% and 1000 wt.% water. For example, materials having hydrophilic or hydrogen bonding groups such as hydroxyls, esters, carboxylates, ketones, amines, amides, sulfates and phosphates. The material can be a fiber that can be formed into a flat shape, including fibers that can pressed together into a web structure or mesh structure. The material can also be a fiber that is formed into a yarn and then woven into a web structure or pressed together into a mesh structure. Without limitation, the porous hydrophilic material can include one or more of one or more of a cellulose, starch, maltodextrin, glycerin, sugar, sucralose, dextrose, gum arabic, cotton, wool, silk, rayon, hemp, spandex/lycra/elastane, polyester, polyamide, linen, nylon, or combinations thereof.

[0073] ***Chamber for holding FDCF biological components***

[0074] Chambers or reaction chambers, wells or sachets are described herein and refer to a space, for example, where the FDCF biological components are disposed, placed or contained. In some implementations, the chamber volume is between about 0.1 μL and about 500 μL, between about 1 and 150 μL, or between about 1 and 100 μL.

[0075] The chambers include a port or small opening (e.g., FIG. 3C port 306, FIG. 4A-4B, FIG. 5A-5B port 410). In some implementations, the port is between 0.1μm^2 and 50 mm^2, such as between 1 μm^2 and 10 mm^2). The port is configured to allow fluid access into the chamber and

in some implementations is not self-sealing. The fluid access should be fast, for example within at least five minutes. In some implementations within 1 minute. In some implementations within at least 30 seconds. In some implementations within 10 seconds, within 5 seconds, or within one second. In some implementations, the port has a cover, for example to seal off the chamber from liquids when the sensor device is not in use, is not usable or when the device may be intentionally exposed to a liquid that is not expected to contain a triggering compound. For example, the user may wish to deactivate the sensor by covering the port before the sensor is immersed in water or when the user is in a wet environment such as in an area with precipitation. The cover can be any form such as a friction fit plug or adhesively attached.

[0076] The sample chambers are impermeable to outside aqueous solutions except through the port opening. The ports are designed for wicking in small volumes, such as from splashes of between with volumes a low as about 1 μL (e.g., between 10 and about 500 μL) at relative humidities between about 20-40%. The chamber and port are also configured to reduce the amount of evaporation once an aqueous solution has entered the chamber. In some implementations the evaporation rate is less than about 1% volume/hr (v/hr). In some implementations, the evaporation rate is less than about 5% v/hr. In some implementations, the evaporation rate is less than about 10% v/hr. In some implementations, the evaporation rate is less than about 15% v/hr. In some implementations, the evaporation rate is less than about 20% v/hr.

[0077] ***Biological components for sensors***

[0078] According to the various aspects, FDCF Biological Components are used as circuits that are triggered by a triggering compound to provide a detectable signal. For example, in some implementations, the synthetic biological components provide the optical signal when activated with the triggering compound by synthesizing, activating, or suppressing, a colored, fluorescent or luminescent protein. In some implementations, the synthetic biological components include toehold sensor components, transcription-factor sensor components, aptameric sensor components, enzyme sensor components, antibody sensor components, CRISPR DNA sensor components, CRISPR RNA sensor components, ribonucleoprotein sensor components, and combinations thereof.

[0079] According to some implementations, the biological components can be supplied from a commercial source. For example, cell-free NEB PUREXPRESS® reaction components (New England Biolabs, Inc., Ipswich, MA). The reaction components, such as an A and a B component are combined and diluted with water to a specified concentration according to the manufactures specification for use. It has been found that using a higher concentration than the specified

concentration range provides faster kinetics according to some implementations of this disclosure. However, at too high a concentration, the signal kinetics of the reaction are negatively impacted. In some implementations, the rehydrated synthetic biological components have a concentration between 1 and 2.4 times a specified concentration. The reaction kinetics are improved using the higher concentrations, as compared to the specified concentrations, by at least 5%, at least 10%, at least 20%, or at least 50%.

[0080] *CRISPR*

[0081] Microbial Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated (CRISPR-Cas) adaptive immune systems contain programmable endonucleases, such as Cas12a Cpf1 (also referred to as Cpf1) and Cas9. Although both Cas12a and Cas9 and target DNA, single effector RNA-guided RNases also have been recently discovered (Shmakov et al., 2015) and characterized (Abudayyeh et al., 2016; Smargon et al., 2017). These programmable endonucleases and RNases provide a platform for specific nucleic acid (DNA or RNA) sensing. DNA-guided endonucleases, such as Cas 12a and Cas9 can be easily and conveniently reprogrammed using CRISPR guide RNA (gRNAs) to cleave target DNAs. RNA-guided RNases, such as C2c2, can be easily and conveniently reprogrammed using CRISPR RNA (crRNAs) to cleave target RNAs.

[0082] Once activated through recognition of the target DNA (e.g., double-stranded DNA) or RNA, many of the CRISPR-Cas endonucleases and RNases exhibit promiscuous non-specific DNase or RNase activity. Thus, after cleavage of the target DNA (e.g., dsDNA) or RNA, the CRISPR-Cas endonucleases and RNases can lead to "collateral" cleavage of any non-targeted DNAs or RNAs present in proximity.

[0083] In general, a CRISPR-Cas or CRISPR system as used in herein refers collectively to transcripts and other elements involved in the expression of or directing the activity of CRISPR-associated ("Cas") genes, including sequences encoding a Cas gene, a tracr (trans-activating CRISPR) sequence (e.g. tracrRNA or an active partial tracrRNA), a tracr-mate sequence (encompassing a "direct repeat" and a tracrRNA-processed partial direct repeat in the context of an endogenous CRISPR system), a guide sequence (also referred to as a "spacer" in the context of an endogenous CRISPR system), or "RNA(s)" as that term is herein used (e.g., RNA(s) to guide Cas, such as Cas12a, e.g. CRISPR RNA and transactivating (tracr) RNA or a single guide RNA (sgRNA) (chimeric RNA)) or other sequences and transcripts from a CRISPR locus. In general, a CRISPR system is characterized by elements that promote the formation of a CRISPR complex at the site of a target sequence (also referred to as a protospacer in the context of an endogenous CRISPR system).

[0084] The CRISPR-Cas effector protein can be from an organism from a genus comprising Streptococcus, Campylobacter, Nitratifractor, Staphylococcus, Parvibaculum, Roseburia, Neisseria, Gluconacetobacter, Azospirillum, Sphaerochaeta, Lactobacillus, Eubacterium, Corynebacter, Carnobacterium, Rhodobacter, Listeria, Paludibacter, Clostridium, Lachnospiraceae, Clostridiaridium, Leptotrichia, Francisella, Legionella, Alicyclobacillus, Methanomethyophilus, Porphyromonas, Prevotella, Bacteroidetes, Helcococcus, Letospira, Desulfovibrio, Desulfonatronum, Opitutaceae, Tuberibacillus, Bacillus, Brevibacilus, Methyl obacterium or Acidaminococcus.

[0085] In some implementations, the effector protein can comprise a chimeric effector protein comprising a first fragment from a first effector protein (e.g., a Cpfl) ortholog and a second fragment from a second effector (e.g., a Cpfl) protein ortholog, and wherein the first and second effector protein orthologs are different. At least one of the first and second effector protein (e.g., a Cpfl) orthologs can comprise an effector protein (e.g., a Cpfl) from an organism comprising Streptococcus, Campylobacter, Nitratifractor, Staphylococcus, Parvibaculum, Roseburia, Neisseria, Gluconacetobacter, Azospirillum, Sphaerochaeta, Lactobacillus, Eubacterium, Corynebacter, Carnobacterium, Rhodobacter, Listeria, Paludibacter, Clostridium, Lachnospiraceae, Clostridiaridium, Leptotrichia, Francisella, Legionella, Alicyclobacillus, Methanomethyophilus, Porphyromonas, Prevotella, Bacteroidetes, Helcococcus, Letospira, Desulfovibrio, Desulfonatronum, Opitutaceae, Tuberibacillus, Bacillus, Brevibacilus, Methyl obacterium or Acidaminococcus; e.g., a chimeric effector protein comprising a first fragment and a second fragment wherein each of the first and second fragments is selected from a Cpfl of an organism comprising Streptococcus, Campylobacter, Nitratifractor, Staphylococcus, Parvibaculum, Roseburia, Neisseria, Gluconacetobacter, Azospirillum, Sphaerochaeta, Lactobacillus, Eubacterium, Corynebacter, Carnobacterium, Rhodobacter, Listeria, Paludibacter, Clostridium, Lachnospiraceae, Clostridiaridium, Leptotrichia, Francisella, Legionella, Alicyclobacillus, Methanomethyophilus, Porphyromonas, Prevotella, Bacteroidetes, Helcococcus, Letospira, Desulfovibrio, Desulfonatronum, Opitutaceae, Tuberibacillus, Bacillus, Brevibacilus, Methyl obacterium or Acidaminococcus wherein the first and second fragments are not from the same bacteria; for instance a chimeric effector protein comprising a first fragment and a second fragment wherein each of the first and second fragments is selected from a Cpfl of S. mutans, S. agalactiae, S. equisimilis, S. sanguinis, S. pneumonia; C. jejuni, C. coli; N. salsuginis, N. tergarcus; S. auricularis, S. carnosus; N. meningitides, N. gonorrhoeae; L. monocytogenes, L. ivanovii; C. botulinum, C. difficile, C. tetani, C. sordellii; Francisella tularensis 1, Prevotella albensis, Lachnospiraceae bacterium MC2017 1, Butyrivibrio

proteoclasticus, Peregrinibacteria bacterium GW2011_GWA2_33_10, Parcubacteria bacterium GW2011_GWC2_44_17, Smithella sp. SCADC, Acidaminococcus sp. BV3L6, Lachnospiraceae bacterium MA2020, Candidatus Methanoplasma termitum, Eubacterium eligens, Moraxella bovoculi 237, Leptospira inadai, Lachnospiraceae bacterium ND2006, Porphyromonas crevioricanis 3, Prevotella disiens and Porphyromonas macacae, wherein the first and second fragments are not from the same bacteria.

[0086] In the context of formation of a CRISPR complex, "target sequence" refers to a sequence to which a guide sequence is designed to have complementarity, where hybridization between a target sequence and a guide sequence promotes the formation of a CRISPR complex. A target sequence can be DNA or RNA. Generally, the term target nucleic acid refers to a polynucleotide being or comprising the target sequence. In other words, the target nucleic acid can be a polynucleotide or a part of a polynucleotide to which a part of the gRNA, i.e. the guide sequence, is designed to have complementarity and to which the effector function mediated by the complex comprising CRISPR effector protein and a gRNA is to be directed.

[0087] It is noted that the effector protein can be a DNA targeting CRISPR-Cas protein or an RNA targeting CRISPR-Cas protein. Exemplary CRSIPR-Cas proteins include, but are not limited to, Casl, CaslB, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Cas9 (also known as Csnl and Csxl2), CaslO, Csyl, Csy2, Csy3, Csel, Cse2, Cscl, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmrl, Cmr3, Cmr4, Cmr5, Cmr6, Csbl, Csb2, Csb3, Csxl7, Csxl4, CsxlO, Csxl6, CsaX, Csx3, Csxl, Csxl5, Csfl, Csf2, Csf3, Csf4, homologues thereof, or modified versions thereof.

[0088] The terms "orthologue" (also referred to as "ortholog" herein) and "homologue" (also referred to as "homolog" herein) are well known in the art. By means of further guidance, a "homologue" of a protein as used herein is a protein of the same species which performs the same or a similar function as the protein it is a homologue of. Homologous proteins can but need not be structurally related, or are only partially structurally related. An "orthologue" of a protein as used herein is a protein of a different species which performs the same or a similar function as the protein it is an orthologue of. Orthologous proteins can but need not be structurally related, or are only partially structurally related. Homologs and orthologs can be identified by homology modelling (see, e.g., Greer, Science vol. 228 (1985) 1055, and Blundell et al. Eur J Biochem vol 172 (1988), 513) or "structural BLAST" (Dey F, Cliff Zhang Q, Petrey D, Honig B. Toward a "structural BLAST": using structural relationships to infer function. Protein Sci. 2013 Apr;22(4):359-66. doi: 10.1002/pro.2225.). See also Shmakov et al. (2015) for application in the

field of CRISPR-Cas loci. Homologous proteins can but need not be structurally related, or are only partially structurally related.

[0089] In some embodiments, the effector protein has a sequence homology or sequence identity of at least 60%, more particularly at least 70, such as at least 80%, more preferably at least 85%, even more preferably at least 90%, such as for instance at least 95%, with the wild-type sequence. The skilled person will understand that this includes truncated forms of effector protein whereby the sequence identity is determined over the length of the truncated form.

[0090] The CRISPR-Cas effector protein can be from an organism from a genus comprising Streptococcus, Campylobacter, Nitratifractor, Staphylococcus, Parvibaculum, Roseburia, Neisseria, Gluconacetobacter, Azospirillum, Sphaerochaeta, Lactobacillus, Eubacterium, Corynebacter, Carnobacterium, Rhodobacter, Listeria, Paludibacter, Clostridium, Lachnospiraceae, Clostridiaridium, Leptotrichia, Francisella, Legionella, Alicyclobacillus, Methanomethyophilus, Porphyromonas, Prevotella, Bacteroidetes, Helcococcus, Letospira, Desulfovibrio, Desulfonatronum, Opitutaceae, Tuberibacillus, Bacillus, Brevibacilus, Methyl obacterium or Acidaminococcus.

[0091] In some implementations, the effector protein is a promiscuous non-specific DNase or RNase such as Cas 9, Cas12a, Cas13a, or Cas14. In some implementations, the effector protein is Cas12a, also known as Cpf1 or Cas 13a, also known as C2c2.

[0092] Selection of a promiscuous non-specific DNase or RNase activity Detections is by addition of a short nucleotide sequence that is coupled to a fluorescent reporter and a quencher. Cleavage of the nucleotide allows separation of the quencher from the fluorescent report providing the detectable signal.

[0093] *SHERLOCK*

[0094] SHERLOCK refers to "Specific High-sensitivity Enzymatic Reporter un-LOCKing." SHERLOCK works by amplifying RNA (or DNA with a reverse transcriptase) using recombinase polymerase amplification (RPA) which is an isothermal nucleic acid amplification. SHERLOCK is useful for biosensors, such as wearable biosensors, because isothermal amplification does not require specialized instrumentation, such as PCR, as it uses a single temperature. The amplified nucleotides are combined with an effector protein (e.g., Cas 13a), a guide RNA that matches the nucleic acid sequence of interest, and a short nucleotide sequence that is coupled to a fluorescent reporter and a quencher. If the target sequence is present in the pool of amplified nucleotides, the non-specific RNAse activity of effector protein becomes activated and the RNA reporter will be cleaved resulting in activation of the fluorophore. Therefore, the fluorescent signal is used as an indicator to determine whether the target sequence

is present in the original pool of nucleotides. Hence the name "Specific high-sensitivity enzymatic reporter unlocking."

[0095] *Light-up aptamers*

[0096] Light-up aptamers are RNA aptamers that bind with their cognate fluorogen ligands and activate their fluorescence. A non-hindered fluorogen can be excited and have its energy dissipated by non-radiative pathway such as molecular vibrations (heat). Once tightly bound by an aptamer, the fluorophore is stabilized and radiative fluorescence decay pathways predominate, leading to a large fluorescence increase. The RNA aptamers can be selected or designed to target specific molecules (trigger compounds) such as small molecules and metabolites. Without the trigger compound, the aptamer region remain unfolded and cannot bind the fluorogen.

[0097] According to some implementations, aptamers MFA, BFR, DIT-Apt1, Spinach, Spinach2, Mango, Broccoli, and dimeric Broccoli can be used. Any congnate fluorgen ligand can be used as the trigger compound. For example, in some implementations, the fluorogen is DFHBI (3,5-difluoro-4-hydroxybenzylidene imidazolinone), Malachite green, Hoechst 1C, DIR, DMHBI, DMABI, 2-HBI, 2-HBI, DFHBI, T01-Biotin, T030Biotin, DFHBI-1T, DFHBI-2T, and PFP-DFHBI.

[0098] *Toehold Switch*

[0099] Toehold switch sensors are synthetic riboregulators that control the translation of a gene via RNA-RNA interactions. They utilize a designed hairpin structure to block gene translation in *cis* by sequestration of the ribosome binding site (RBS) and start codon. Translation is activated upon the bindig of a *trans*-acting trigger RNA to the toehold region of the switch, which relieves the sequestration of the RBS and allows translation of the down-stream gene. Toehold switch sensors can be designed to bind nearly any RNA sequence. The switch output is described below.

[00100] *Transcription-factor switch*

[00101] Transcriptional factor based biosensors consist of a repressor or activator protein regulating the transcriptional activity of a specific promoter. A cis-regulatory DNA sequence (generally called operator or enhancer) adjacent to the promoter is the core DNA element that binds with a TF restricting or enhancing the access of RNA polymerase (RNAP) to the promoter. A repressor binds to the operator and prevents RNAP proceeding forward to decrease transcription an activator binds to the enhancer elements and promotes the formation of more stable RNAP-promoter complex to increase transcription. Apart from the DNA-binding domain, TFs also contain a ligand-binding domain which is the sensor domain that responds to small molecules or environmental stress signal (salt, osmosis, pH, oxygen, redox, light or radiation

etc.). Transcriptional activators can be any activators that are coupled to the specific repressor or activator protein used. In some implementations the transcriptional activators are VP 16, VP 64, FapR, FdeR, PcaQ, ArgP, MdcR, Yap1, Gal4, TetR, TrpR, FadR, PhlF, or LexA. The switch output is described below.

[00102] *Riboswitch*

[00103] Riboswitches are RNA-based sensors that utilize chemically induced structural changes in the 5′-untranslated region of mRNA to regulate expression of downstream genes. Riboswitches are quickly synthesized in vitro, flexible in engineering (both aptamers and expression platforms), and can provide a fast response to recognize elements due to the avoidance of complicated protein–protein interactions, even before considering their high specificity and sensitivity. In some implementations, a typical riboswitch construct includes two domains linked to each other, a sensory domain and the regulatory domain. The aptamer binds to the target ligand and causes sufficient conformational changes or stability changes which then trigger the desired readout in the expression platform (switch output) through different mechanisms depending on the choice of expression control at the translation or transcription level. The switch output is described below.

[00104] *Switch output*

[00105] In some implementations, the switch output can be expression of any protein providing colorimetric, fluorescent or phosphorescent output. In some implementation the protein is selected from one or more of GFP, LacZ, Luciferase, TagBFP, mTagBFP2, Azurite, EBFP2, mKalama1, Sirius, Sapphire, or T-Sapphire; cyan proteins: ECFP, Cerulean, SCFP3A, mTurquoise, mTurquoise2, monomeric Midorishi-cyan, TagCFP, mTFP1, EGFP, Emerald, Superfolder GFP, Monomeric Azami Green, TagGFP2, mUKG, mWasabi, Clover, mNeonGreen, EYFP, Citrine, Venus, SYFP2, TagYFP, Monomeric Kusabira-Orange, mKOκ, mKO2, mOrange, mOrange2, mRaspberry, mCherry, mStrawberry, mTangerine, tdTomato, TagRFP, TagRFP-T, mApple, mRuby, mRuby2, far-red proteins; mPlum, HcRed-Tandem, mKate2, mNeptune, NirFP, near-IR proteins; TagRFP657, IFP1.4, iRFP, long stokes shif proteins; mKeima Red, LSS-mKate1, LSS-mKate2, and mBeRFP. In some implmentaions the protein is GPF, LacZ, luciferase or combinations of these.

[00106] Green Fluorescent Protein (GFP) is a single polypeptide gene product of 238 amino acids discovered in the jellyfish Aequorea victoria. The protein has a natural green fluorescence. GFP is quite stable and withstands a number of chemical treatments and procedures. GFP requires no biochemical transformation, contrast agent or the use of harmful ionizing radiation in order to be visualized. Enhanced GFP (EGFP) has been engineered to be expressed at higher

levels in mammalian cells and to fluoresce more intensely. Cyan Fluorescent Protein (CFP) and Yellow Fluorescent Protein (YFP) are spectral variants of GFP that allow multiple cell types to be labeled simultaneously.

[00107] The *lacZ* gene encodes beta-galactosidase, which catalyzes the cleavage of lactose to form galactose and glucose. Beta-galactosidase activity can be identified by when incubated with the beta-galactosidase substrate X-gal. Beta-galactosidase cleaves X-gal, a chromogenic substrate, resulting in an insoluble blue dye, thus allowing for the identification of *lacZ* activity.

[00108] Luciferase are a class of oxidative enzymes that produce bioluminescence. Luciferase enzymes isolated from different animal species have inherent variability in light emission. For example, Luciferase enzymes are commercially available from the organisms *Photinus pyralis, Luciola cruciate, Luciola italic, Luciola lateralis, Luciola mingrelica, Photuris pennsylvanica, Pyrophorus plagiophthalamus, Phrixothrix hirtus, Renilla reniformis, Gaussia princeps, Cypridina noctiluca, Cypridina hilgendorfii, Metridia longa,* and *Oplophorus gracilorostris*.

[00109] ***Triggering compounds***

[00110] The triggering compounds (e.g., trigger, or trigger compound) can be any compound for which the synthetic biology switch is designed or selected. In some implementations the triggering compound can include natural or synthetic molecules including, but not limited, peptides, oligonucleotides polypeptides, proteins, peptidomimetics, antibodies, antibody fragments (e.g., antigen binding fragments of antibodies), carbohydrate-binding protein, e.g., a lectin, glycoproteins, glycoprotein-binding molecules, amino acids, carbohydrates (including mono-, di-, tri- and poly-saccharides), lipids, steroids, hormones, lipid-binding molecules, cofactors, nucleosides, nucleotides, nucleic acids (e.g., DNA or RNA, analogues and derivatives of nucleic acids, or aptamers), peptidoglycan, lipopolysaccharide, small molecules, and any combinations thereof.

[00111] As used herein, the term “small molecules" refers to natural or synthetic molecules including, but not limited to, amino acids, peptides, peptidomimetics, polynucleotides, aptamers, nucleotide analogs, organic or inorganic compounds (i.e., including heterorganic and organometallic compounds), saccharides (e.g., mono, di, tri and polysaccharides), steroids, hormones, pharmaceutically derived drugs (e.g., synthetic or naturally occurring), lipids, derivatives of these (e.g., esters and salts of these), fragments of these, and conjugates of these. In some implementations the small molecules have a molecular weight less than about 10,000 Da, organic or inorganic compounds having a molecular weight less than about 5,000 Da, organic or inorganic compounds having a molecular weight less than about 1,000 Da, organic or

inorganic compounds having a molecular weight less than about 500 Da. In some implementations the small molecule has a molecular weight of less than about 1000 Da.

[00112] In some implementations, the triggering compound can include aptamers. As used herein, the term "aptamer" means a single-stranded, partially single-stranded, partially double-stranded or double-stranded nucleotide sequence capable of specifically recognizing a selected non-oligonucleotide molecule or group of molecules by a mechanism other than Watson-Crick base pairing or triplex formation. Aptamers can include, without limitation, defined sequence segments and sequences comprising nucleotides, ribonucleotides, deoxyribonucleotides, nucleotide analogs, modified nucleotides and nucleotides comprising backbone modifications, branchpoints and nonnucleotide residues, groups or bridges. The oligonucleotides including aptamers can be of any length, e.g., from about 1 nucleotide to about 100 nucleotides, from about 5 nucleotides to about 50 nucleotides, or from about 10 nucleotides to about 25 nucleotides.

[00113] In some implementation the triggering compound is a component that is extracted or lysed from a microbe. As used interchangeably herein, the terms "microbes" and "pathogens" generally refer to microorganisms, including bacteria, fungi, protozoan, archaea, protists, e.g., algae, and a combination thereof. The term "microbes" also includes pathogenic microbes, e.g., bacteria causing diseases such as plague, tuberculosis and anthrax; protozoa causing diseases such as malaria, sleeping sickness and toxoplasmosis; fungi causing diseases such as ringworm, candidiasis or histoplasmosis; and bacteria causing diseases such as sepsis. The term "microbe" or "microbes" can also encompass non-pathogenic microbes, e.g., some microbes used in industrial applications. In some implementations, the term "microbe" or "microbes" also encompasses fragments of microbes, e.g., cell components of microbes, LPS, and/or endotoxin.

[00114] In some implementations the trigger molecule is a "molecular toxin," which refers to a compound produced by an organism which causes or initiates the development of a noxious, poisonous or deleterious effect in a host presented with the toxin. Such deleterious conditions may include fever, nausea, diarrhea, weight loss, neurologic disorders, renal disorders, hemorrhage, and the like. Toxins include, but are not limited to, bacterial toxins, such as cholera toxin, heat-liable and heat-stable toxins of *E. coli*, toxins A and B of *Clostridium difficile*, aerolysins, and hemolysins; toxins produced by protozoa, such as *Giardia*; toxins produced by fungi. Molecular toxins can also include exotoxins, i.e., toxins secreted by an organism as an extracellular product, and enterotoxins, i.e., toxins present in the gut of an organism.

[00115] ***Lysate***

[00116] According to some implementations a lysate, e.g., a prokaryotic or a eukaryotic cell lysate is used. The lysate can be combined with the FDCF biological components prior to contact

with an aqueous solution, or the lysate can be first combined with the aqueous solution. In some implementations, the lysate includes one or more of Triton X-100, NP-40, Tween-20, Brij non-ionic surfactants, CHAPS hydrate, lysozyme, and disaccharides or polysaccharides such as sucrose, mannitose, or trehalose. In some implementations, the lysate is freeze-dried. In some implemenations the lysate is dried by another method, such as by evaporating the solvent above the freezing temperature (e.g., under vacuum). In some implementations, the lysate does not include a cationic surfactant. In some implementations, the amount of ionic surfactant by weight of total dry lysate is less than about 20%, less than about 10%, less than about 5%, or less than about 1%.

[00117] ***Dissolvable membranes or dissolvable materials***

[00118] In some implmentations a dissolvable membrane can be integrated into a sensor, for example, in order to allow control of sample flow. The membrane acts as a time-barrier film, by stopping the sample flow until it is dissolved. The control of sample flow in sensor critical areas, such as cell lysing regions, allows increased exposure time for the lysing reagents to act. This helps to ensure higher sensitivity, reactivity and in some cases reduces false-positive signals.

[00119] Dissolvable membranes contain a water-soluble polymer, sugars such as sucrose, inorganic salts, patterned hydrophobic materials, or other compounds to provide a fluidic delay. In some implmentations the water-soluble polymer is a hydroxylpropyl-methylcellulose, polyvinylpyrrolidone, polyvinyl-alcohol (PVA), carboxymethyl-cellulose, polyethylene-oxide, hydroxylpropyl-cellulose, hydroxylethyl-cellulose, methyl-cellulose, pullulan, gelatin, pectin, sodium alginate, maltodextrin, polymerized rosin, and xanthan. In some implementations a plasticizer is added, for example, to improve mechanical properties such as brittelness. In some implmentations the plasticizers is glycerol, propylene glycol, poly (ethylene glycol), glycerine, dimethyl phthalate, diacetyl phthalate, dibutyl phthalate, triacetrin, castor oil, citrate ether, and tryethyle citrate.

[00120] ***Blocking agents***

[00121] As used herein a “blocking agent” or “molecular blockers” are compounds used to prevent non-specific interactions. The blocking agent can be a coating on a surface, e.g., of the substrate, that prevents non-specific interactions or fouling of the surface when it is contacted with the test sample. A blocking agent includes a compound that either covalently bonds with the material it is blocking or uses non-covalent interactions to block the material with a desired physiochemical characteristic. Blocking agents can be used to treat any surfaces and materials described herein. In some implementations, the interior or exterior surfaces of sensors are treated with blocking agents. In some implementation, the porous or non-porous hydrophilic materials

are treated with blocking agents. In some implementations, hydrophobic materials such as elastomers are treated with blocking agents.

[00122] Non-specific interactions can include any interaction that is not desired between the target molecule (e.g., a triggering compound) and the surface (e.g., a porous hydrophilic material) or between other components in solution. The blocking agent can be a protein, mixture of proteins, fragments of proteins, peptides or other compounds that can passively absorb to the surface in need of blocking. For example, proteins (e.g., BSA and Casein), poloxamers (e.g., pluronics), PEG-based polymers and oligomers (e.g., diethylene glycol dimethyl ether), cationic surfactants (e.g., DOTAP, DOPE, DOTMA). Some other examples include commercially available blocking agent or components therein that are available from, for example, Rockland Inc. (Limeric, PA) such as : BBS Fish Gel Concentrate; PBS Fish Gel Concentrate; TBS Fish Gel Concentrate; Blocking Buffer for Fluorescent Western Blotting; BLOTTO; Bovine Serum Albumin (BSA); ELISA Microwell Blocking Buffer; Goat Serum; IPTG (isopropyl beta-D-thiogalactoside) Inducer; Normal Goat Serum (NGS); Normal Rabbit Serum; Normal Rat Serum; Normal Horse Serum; Normal Sheep Serum; Nitrophenyl phosphate buffer (NPP); and Revitablot™ Western Blot Stripping Buffer. In some implmentations, the blocking agent is BSA.

[00123] In some embodiments, the blocking agent can be a monomer. In general, a monomer (with a single binding site) has no free binding site after binding to the target-binding agent. For example, saccharide-based monomeric blocking agent. In some embodiments the blocking agent can be a monosaccharide or modification thereof, including, e.g., but not limited to, diose, triose, tetrose, pentose, hexose, heptose, linear chain monosaccharides, open chain monosaccharides, cyclic isomers (e.g., furanose form and pyranose of monosaccharides such as hexose), pyranose, fructose, galactose, xylose, ribose, amino sugars (e.g., but not limited to, galactosamine, glucosamine, sialic acid, N-acetylglucosamine, N-acetyl-muramic acid, sulfosugars (e.g., but not limited to sulfoquinovose).

[00124] ***Biological fluids***

[00125] In some implementations, the aqueous solution can include a biological fluid. Exemplary biological fluids can include, but are not limited to, blood (including whole blood, plasma, cord blood and serum), lactation products (e.g., milk), amniotic fluids, sputum, saliva, urine, semen, cerebrospinal fluid, bronchial aspirate, perspiration, mucus, liquefied stool sample, synovial fluid, lymphatic fluid, tears, tracheal aspirate, and any mixtures thereof. In some embodiments, a biological fluid can include a homogenate of a tissue specimen (e.g., biopsy). In one embodiment, an aqueous solution is a suspension obtained from homogenization of a solid sample obtained from a solid organ or a fragment thereof.

[00126] ***Optical fibers***

[00127] In some implementations optical fibers are used to transmit excitation or emissions. Optical fibers are waveguide fibers designed for transmission of light. Optical fibers typically include a core surrounded by a transparent cladding material with a lower index of refraction. Light is kept in the core by total internal reflection. Optical fibers can include glass (silica, fluorozirconate, fluoroaluminate, and chalcogenide glasses) or plastic optical fibers (POF). Glass optical fiber can be made having a high fidelity and low transmission loss, and are often regarded as the fiber of choice for many optical applications, such as communications and long range transmission. For low speed short data links, POFs can often be implemented. POFs also have the advantage of being more flexible than glass optical fibers. POFs are also more economical and the optical fiber of choice for many consumer products, such as digital home appliance networks, home networks and car networks. Being flexible, POFs are rugged and easy to install without fear of damage. POFs generally have a diameter about 8 times that of glass optical fibers.

[00128] In some implmentations the optical fiber is a POF. For example, having a poly methyl methacrylate core, or polystyrene core, and having a fluorinated polymer or silicone resin cladding. In some implementations the POFs have a diameter between about 2000μm and 200 μm, between about 1500 μm and 500 μm, or between about 1200 and about 800μm.

[00129] In some implementations, one end of the POF is coated with a reflective coating. The coatings ensure light that would escape from the end that is not connected to an emission source or to the detector is not lost. Any reflective coating can be used that reflects at least about 10% of incident light (e.g., at least 20%, at least 50%, at least 80%). For example, reflective coatings can include a metal coating such as gold and silver.

[00130] ***Wearable Items***

[00131] The sensors described herein can be configured as, although not limited to, a wearable item. Without limitation these can include a shirt; a jacket; pants; a skirt; a laboratory coat; a full-body garment; an exterior worn armor; a wrist, arm, head or ankle band; a scarf; gloves; socks; shoes or boots; a necklace; a ring; a hat; a helmet; a brooch; a face mask; a patch; or other wearable garments.

[00132] It should be understood that this invention is not limited to the particular methodology, protocols, and reagents, etc., described herein and as such may vary.

[00133] In one aspect, the present invention relates to the herein described compositions, methods, and respective component(s) thereof, as essential to the invention, yet open to the

inclusion of unspecified elements, essential or not ("comprising"). In some embodiments, other elements to be included in the description of the composition, method or respective component thereof are limited to those that do not materially affect the basic and novel characteristic(s) of the invention ("consisting essentially of"). This applies equally to steps within a described method as well as compositions and components therein. In other embodiments, the inventions, compositions, methods, and respective components thereof, described herein are intended to be exclusive of any element not deemed an essential element to the component, composition or method ("consisting of").

[00134] As used herein, the term “small molecules" refers to natural or synthetic molecules including, but not limited to, peptides, peptidomimetics, amino acids, amino acid analogs, polynucleotides, polynucleotide analogs, aptamers, nucleotides, nucleotide analogs, organic or inorganic compounds (i.e., including heteroorganic and organometallic compounds) having a molecular weight less than about 10,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 5,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 1,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds.

EXAMPLES

[00135] The following examples illustrate some embodiments and aspects of the invention. It will be apparent to those skilled in the relevant art that various modifications, additions, substitutions, and the like can be performed without altering the spirit or scope of the invention, and such modifications and variations are encompassed within the scope of the invention as defined in the claims which follow. Other than in the operating examples, or where otherwise indicated, all numbers expressing quantities of ingredients or reaction conditions used herein should be understood as modified in all instances by the term “about.” The following examples do not in any way limit the invention.

[00136] *Wearable Freeze Dried Cell Free Sensors*

[00137] Colorimetric genetic circuits were embedded into cellulose substrates surrounded by a fluid wicking and containment assembly made of flexible elastomers (FIG. 6A). These prototypes were assembled layer-by-layer to form reaction chambers fluidly connected to top sample portals (FIGs. 6B, 7A). The devices are flexible, elastic, and can rapidly wick in splashed fluids through capillary action (FIGs. 6C,6D). Pinning geometries throughout the device direct

sample fluids towards enclosed hydrophilic paper networks allowing for reaction rehydration (FIGs. 6B and 8B). Using a *lacZ* β-galactosidase operon as the circuit output to hydrolyze chlorophenol red-β-D-galactopyranoside (CPRG), a yellow to purple color change develops upon exposure to a target (FIGs. 7B, 8A).

[00138] Key environmental factors were considered for the design of these prototypes. For instance, sample exposure in the field likely occurs with variable splash volumes (as little as 50-100 μL), relative humidity (RH, 20-40%), and temperature (20-37°C). Thus, the design was optimized to reduce inhibition of genetic circuit operation due to evaporation or excessive dilution of components. In particular, the devices use impermeable chambers exhibiting low evaporation rates (<20% volume/hour), which also constrains the rehydration volume to ~50 μL per sensor. In addition, the wFDCF reactions were optimized to generate a higher concentrated reaction upon rehydration. It was found that a 1.5x-concentrated cell-free reaction increased the reaction kinetics to enable signal output at least 10 min faster, ensuring that the desired circuit is completed before eventual evaporation in the device terminates the reaction (FIG. 10A-10D). The resulting stand-alone colorimetric system is modular and can be used in garments such as bracelets (FIG. 8C).

[00139] Functional testing of this colorimetric wearable platform was performed utilizing four different synthetic biology biosensors with *lacZ* as the output (FIGs. 6E-6H). These various demonstrations include a constitutive *lacZ* expression reaction (FIG. 6E), a transcription factor-regulated circuit using the tetracycline repressor (TetR) (FIG. 6F), a toehold switch for Ebola virus RNA detection (FIG. 6G), and a theophylline riboswitch for small-molecule sensing (FIG. 6H). Genetic circuits using transcriptional regulators are among some of the most common elements used in synthetic biology. The wFDCF TetR sensor demonstrates the capacity of the colorimetric platform for facile integration of well-established genetic modules into a wearable format (FIG. 6F). Similarly, toehold switches have been developed as highly programmable nucleic acid sensors capable of detecting any target RNA. It was shown that a wFDCF Ebola virus RNA toehold sensor in the wearable device is capable of rapid and sensitive detection of biothreats (FIG. 6G). Similar viral or bacterial wearable nucleic acid sensors can be made. Furthermore, a functional theophylline riboswitch wFDCF circuit is functionally validated in these platforms for the environmental detection of small molecules via engineered cis-regulated RNA circuits (FIG. 6H). This specific riboswitch was selected as a model test case, although a plethora of similar riboswitches for various targets have been reported and can be used in a modular fashion. All of the colorimetric wFDCF sensors reported here exhibited visible changes within ~40-60 min after exposure to the respective trigger molecules or inducer, and were

performed at ambient conditions of 30-40% RH and 30°C to simulate the average skin surface temperature.

[00140] Expanding on the attractiveness and versatility of textiles as ubiquitous wearable substrates, FDCF synthetic biology systems within wearable woven fabrics and individual threads were made. FIGs. 9A-9G presents various demonstrations of a highly sensitive, textile-based system (FIGs. 9A, 9B) capable of containing and monitoring the activation of wFDCF reactions with fluorescent (FIGs. 9C-9E, 11-14) or luminescent (FIGs. 9F, 15A-15B) outputs. To achieve this, a second wearable platform was made that integrates: (a) hydrophilic threads (85% polyester / 15% polyamide) for cell-free reagent immobilization, (b) patterns of skin-safe hydrophobic silicone elastomers for reaction containment, and (c) inter-weaved polymeric optic fibers (POFs) for signal interrogation (FIGs. 9A, 9B, 16A-16D, 17A-17G). This fabric was chosen as the main immobilization substrate after conducting a compatibility screening of over 100 textiles (e.g., silks, cotton, rayon, linen, hemp bamboo, wool, polyester, polyamide, nylon, and combination materials) using a lyophilized constitutive *lacZ* cell-free reaction FIGs. 18A, 18B, 19A, 19B, 20). The analysis of sensor outputs was done using a custom-built wearable POF spectrometer (FIGs. 9B, 21A-21F) that could be monitored with a mobile phone application (FIGs. 22A-22C). Using this integrated platform, distributed on-body sensing of various target exposures was performed, as shown in FIGs. 9C-9F. A sample activation through fluid splashing can be seen in FIG. 9A, where the sample wicks through the entry ports with blackout fabrics to rehydrate the freeze-dried, cell-free synthetic biology reactions immobilized within the hydrophilic textile fibers. These fibers are located within the excitation and emission layers of the device as shown in FIG. 9A, 9B. Trigger presence in the splash fluid leads to activation of the sensor circuits, which produce fluorescent or luminescent reporters.

[00141] The versatility of this textile platform in fluorescence mode was first verified using two independent synthetic biology modules upstream of a superfolder green fluorescent protein (sfGFP) operon. These demonstrations included the activation of constitutive sfGFP expression (FIG. 9C) and sensing of theophylline using an inducible riboswitch (FIG. 9D). A third fluorescence demonstration was done via activation of a 49-nucleotide Broccoli aptamer (FIG. 9E) with substrate-specificity to *(Z)-4-(3,5-difluoro-4-hydroxybenzylidene)-1,2-dimethyl-1H-imidazol-5(4H)-one* (DFHBI-1T), evincing functionality of this emerging class of fluorescent sensors in synthetic biology. Furthermore, demonstrations utilizing luminescence outputs were conducted using a nanoLuciferase operon downstream of an HIV RNA toehold switch (FIG. 9F, 15A), as well as a *B. burgdorferi* RNA toehold switch for the wearable detection of Lyme disease (FIG. 15B).

[00142] Additionally, the operation of this platform was tested for the detection of chemical threats such as organophosphate nerve agents used in chemical warfare and the pesticide industry, both of which constitute prime targets for wearable detection. To achieve this, the POF platform optics for excitation and detection at near-infrared (NIR) fluorescence, generated from a lyophilized acetylcholinesterase (AChE)-choline oxidase (ChOx)-HRP coupled enzyme reaction (FIG. 9G), were modified. In the presence of acetylcholine, this reaction can produce NIR fluorescence that is readily detectable with the wearable prototype. When exposed to an organophosphate AChE inhibitor, the sensor fluorescence is ameliorated as compared to unexposed controls. The wearable nerve agent sensor was validated using paraoxon-ethyl as a nerve agent simulant at levels that are four orders of magnitude lower than the reported lethal dose (LD_{50}) by dermal absorption in mammals (FIG. 9G).

[00143] Despite the single-activation nature of the wFDCF synthetic biology sensors, the presence of fluorescence outputs is continuously monitored to allow for automatic detection of rehydration events containing the desired target. This is achieved by illuminating the wFDCF textile reaction with blue light (447 nm) via etched excitation POFs (FIGs. 9B, 17A). The light emitted from the activated system is then collected by the second set of emission POFs, which exit the fabric weave and bundle into a connection to the optical sensor (FIG. 9B) of the wearable spectrometer (FIGs. 17A-17G, 21A-21F). Signals coming from each of the devices are filtered (FIGs. 17D) and processed to generate temporally and spatially resolved fluorescence images of the POF bundle-ends (510 nm) and averaged pixel intensity traces per channel for quantitative analysis (FIG. 9B). In the case of luminescence demonstrations, all POFs bundles are treated as signal inputs, without the need for sample illumination. All reported wFDCF fluorescence and luminescence sensor replicates ($n \geq 3$) exhibited visible fluorescence or luminescence within 5-20 min after exposure to relevant trigger conditions, at 30-40% RH and 30°C.

[00144] Recent advances in programmable clustered regularly interspaced short palindromic repeat (CRISPR) and CRISPR-associated (Cas) enzymes have enabled the development of new classes of rapid and reliable sensing platforms. The advantages of CRISPR-based systems over existing biosensors include high sensitivity, rapid output, single base-pair resolution, freeze-drying compatibility, and the notable programmability to target any DNA or RNA sequence through interchangeable guide RNAs (gRNAs). Thus, CRISPR-based sensors were integrated into the fluorescence wFDCF platform to demonstrate this detection technique in wearable applications (FIG. 23A). Cas13a and Cas12a were used for the detection of RNA and DNA, respectively. For DNA detection, Cas12a ortholog from *Lachnospiraceae* bacterium

(LbaCas12a) was used that displays a non-specific collateral cleavage activity towards single-stranded DNA (ssDNA) after detection of a gRNA-defined double-stranded DNA (dsDNA) target. This Cas12a-based sensor was paired with recombinase polymerase amplification (RPA) and freeze-dried into a one-pot reaction to demonstrate state-of-the-art detection limits for wearable clinical applications. In the presence of a target dsDNA sequence, isothermally generated RPA amplicons activate Cas12a-gRNA complexes. Then, active Cas12a engages in trans-ssDNase activity and cleaves quenched ssDNA fluorophore probes, resulting in a fluorescence output (FIG. 23A). For the wearable CRISPR-based demonstrations, gRNAs were designed against three common resistance markers in *Staphylococcus aureus*: specifically, the *mecA* gene common in methicillin-resistant *S. aureus* (MRSA), the *spa* gene which encodes the protein A virulence factor, and the *ermA* gene conferring macrolide resistance. When tested in wFDCF format, the RPA-Cas12a sensors displayed detectable signals within 56-78 min ($P<0.05$) with femtomolar limits of detection (FIGs. 23B-23D). Moreover, using the *mecA* wFDCF sensor (FIG. 23E, 24), it was possible to confirm single-digit femtomolar sensitivity (2.7 fM). Compatibility with RNA inputs and other CRISPR enzymes such as Cas13a, an ortholog from *Leptotrichia wadei* bacterium (LwaCas13a) was also confirmed (FIG. 24), exhibiting similar in-device activation dynamics as that of cell-free reactions conducted in a plate reader. These results suggest that the wearable textile platform could be adapted to achieve sensitivities rivaling that of current laboratory diagnostic tests such as qPCR for monitoring contamination or spread of bacteria and viruses.

[00145] To further demonstrate the modularity of the CRISPR-Cas12a wearable sensors, wFDCF devices containing three orthogonal Cas12a-gRNA complexes in isolated reaction chambers were tested (FIG. 23F). In this experiment, each device was splashed with dd-H_2O containing different targets, each specific to only one Cas12a-gRNA complex. The orthogonal behavior of the CRISPR-based wearable sensors is shown in FIGs. 23G-23H, where higher fluorescence was observed for the cases in which the dsDNA trigger matched the pre-defined Cas12a-gRNA complex at each sensor location. These results suggest the broad applicability of CRISPR-based synthetic biology sensors for multiplexing or logic-gating in wearable synthetic biology applications.

[00146] The wFDCF reactions and networked optical fiber detection system can be integrated into flexible textiles to create an autonomous wearable platform enabling real-time monitoring of environmental exposure and biohazard detection. A jacket was designed that contained a distributed arrangement of wFDCF multi-sensor arrays (FIG. 23I). The various optical fibers carrying the output emission signals can be routed into a single bundle for centralized imaging

analysis or interrogated as separate modules, which was demonstrate using a wFDCF CRISPR-Cas12a based MRSA-sensing array containing spa, ermA and mecA sensors that was activated in the wearable prototype with a fluid splash containing 100 fM of spa DNA trigger (FIG. 12). Only the well containing the spa sensor generated a fluorescent signal upon activation. The platform is also compatible with transcription-only outputs, such as rehydrated fluorescent aptamer reactions (FIG. 13), where the fluorescence signal is monitored by microscopy over time.

[00147] In addition, the optical sensor allows for facile fluorescent output multiplexing simply by using fluorescent proteins with orthogonal emission profiles (FIG. 14). In this example, wFDCF reactions for three constitutively expressed fluorescent output proteins (eforRed, dTomato, and sfGFP) were used to demonstrate detection of distinguishable output signals in a single bundle. It is possible that additional fluorescent outputs, including orthogonal quenched fluorophore probes for SHERLOCK-based sensors, can be employed to increase the signal multiplexing of this wearable platform. It is also shown that the wFDCF POF system is fully compatible with integrated lyophilized lysis components, allowing for the release and detection of a plasmid-borne *mecA* gene when challenged with intact bacterial cells (FIGs. 26A-26D). Finally, to develop a complete data feedback cycle between the platform and the user, the detector system was integrated with a custom wireless mobile application that enables continuous cloud-based data logging, signal processing, geolocation tracking, and on-the-fly control of various detector components through a smart phone or other networked digital device (FIG. 23J). All images and spectral data presented in FIGs. 9A-9G, 23A-23J were collected and processed using wFDCF devices fully integrated with the wearable spectrometer and mobile phone application. Further details on the hardware (FIGs. 21A-21F) and software design (FIGs. 22A-22C), as well an implementation of a novel *Opuntia microdasys* bioinspired fluid collection add-on for improved sample harvesting and routing splashes outside of the sensor zones into the wFDCF modules (FIGs. 27A-27D).

[00148] The wearable synthetic biology sensors demonstrated here thus imbue programmable and highly sensitive diagnostic sensing to protective apparel. With the current SARS-CoV-2 pandemic that has led to significant strain on the medical system of all impacted countries and considerable delays in diagnostic testing, the wFDCF system are adapted to key wearable gear, face masks, that have been shown to be critical in reducing the transmission of this highly infectious virus.

[00149] The wFDCF platform are complementary to cell-based synthetic biology sensors. Such living sensors are capable of self-replication, can operate continuously to provide dynamic sensing, and they can actively draw upon environmental resources for energy. However, storage and biocontainment concerns limit their use for wearable technologies. Herein is demonstrated that cell-free synthetic biology systems can be used to build practical wearable biosensors that are shelf-stable, genetically programmable, and highly sensitive.

[00150] The wFDCF sensors are responsive to external rehydration events, such as splashes with contaminated fluids, and withstand inhibitory evaporative and dilutive effects in open-environment conditions (30-40% RH and ~25-30°C). These freeze-dried systems generate measurable colorimetric, fluorescence, or luminescence outputs upon exposure to relevant real-world targets. In the wFDCF POF sensors, continuous monitoring enables rapid alert to an exposure event. The integration of these device designs into garments that are compatible with wireless sensor networks to provide real-time dynamic monitoring of exposure using custom smartphone applications is also demonstrated. Although laboratory testing may be more sensitive, the wFDCF sensors have the distinct advantages of a wearable format, autonomous functioning, and rapid results.

[00151] The presented platform is the first wearable technology demonstrated to detect nucleic acids from potential viral or bacterial pathogens in contaminant fluid samples with sensitivities rivaling those of traditional laboratory tests at ambient temperatures. The wFDCF platform evinces a number of distinct advantages over existing POC diagnostics, which similarly attempt to eliminate the need for time-consuming laboratory tests. Current field-portable POC systems typically use a swabbed or directly applied sample to provide a readout. In contrast to a batch-mode POC sensor, the wFDCF synthetic biology sensors can be networked to provide sensing arrays of lyophilized reactions and lightweight polymer fabrics, thus cloaking the user and continuously generating high-density, real-time outputs without sacrificing comfort or agility in the field. The platform is also designed to operate autonomously, unlike most current POC instruments that require training for use and multiple operations by the user to acquire the final results. This feature removes the need to perform regular exposure checks, freeing those in the field to focus on their core tasks. In comparison to current wearable sensors that primarily employ electronic devices to monitor physiological signals such as heart rate or blood oxygen levels, these modular wearable sensors can detect environmental threats or patient samples through nucleic acid, protein, or small molecule detection. Although recently electrochemical sensors have been integrated into a wearable format, they only detect chemicals and an easily programmable wearable form for sensitive nucleic acid detection does not exist to date. Finally,

the wFDCF components are inexpensive, with cell-free reactions costing only $0.01-0.03 per μL. Thus, a single 10 mm-diameter sensor would currently only cost ~$1 in reagents. The optical fiber textiles are woven from common polymer fibers, and are also inexpensive. At these price points, the wearables could be utilized as disposable protective garments with advanced sensing technology. The sensors are also highly modular and adapted to various form factors, such as clothing.

[00152] Field applications that would greatly benefit from these wFDCF synthetic biology platforms include soldiers and first-responders (e.g., Hazmat personnel, Firemen) operating in environments where a specific chemical or biological threat is suspected. In this situation, the apparel of disposable wFDCF sensors could be used to maintain situational awareness, with continuous spatio-temporal monitoring of exposure and bodily resolution down to centimeters. Another set of potential uses for this platform involves the environmental awareness of clinicians, health workers, and researchers working in high-risk areas. The wearable sensing platforms could enable rapid responses to contagion so that any exposed users could begin decontamination and neutralization procedures immediately. Similarly, wFDCF-enabled coats and gowns in hospitals could provide alerts to prevent the spread of nosocomial infections to vulnerable populations, such as immune-compromised patients or newborns. An additional promising application is patient-worn sensor-enabled wearables such as the face mask presented here that can provide inexpensive, shelf-stable, and labor saving POC diagnostics to rapidly inform clinicians in outbreak events, such as the current COVID-19 pandemic that has rapidly overwhelmed the resources of worldwide medical infrastructures. In another implementation, any animal, such as mammals, can use the wFDCF. For example, a dog associated with a soldiers and first responders can be deployed with or separated with the associated human. In yet other implementations, the wFDCF can be attached to a robot sent in a hazardous environment. In any of the implemenations, the wFDCF can be taken off (e.g., the human, dog, robot) and left to collect and relay or monitor a specific chemical or biological threat.

[00153] *Fabrication of colorimetric synthetic biology wearable modules.*

[00154] Translucent (FIG. 6B top) and opaque (FIG. 6B middle/bottom) layers were made using skin-safe ECOFLEX® silicone elastomer (Smooth-On, Inc, Macungie, PA), precast overnight and laser-cut on a 75W Epilog Legend 36EXT according to the layouts shown in FIG. 6B and 7A. After laser-cutting, the silicone pieces were placed in a warm wash (45°C) with TERGAZYME® detergent (Alconox, Inc., White Plains, NY) for one hour with agitation, followed by three washes in 18-Ω pure water and a final wash in 70% ethanol, before allowing

them to air dry. Layers were aligned and bonded together by depositing freshly-made, uncured liquid silicone elastomer and post-curing overnight at 65°C in a well-ventilated oven to obtain the final assembled prototypes. The final assembled elastomer prototypes were thoroughly sprayed with RNase Away Decontaminant (Thermo Fisher Scientific, Waltham, MA) and washed with 70% ethanol twice before being stored in petri dishes.

[00155] For the support matrices housing the cell-free reactions, clean WHATMAN™ No. 4 filter-paper disks (GE Healthcare Lifesciences Inc., Chicago, IL) (FIG. 6B) were punched to obtain cellulose discs with dimensions of 8 mm diameter and 0.5 mm thickness. These disks were incubated overnight in 0.01% DEPC, washed 3x with nuclease-free water, then incubated with 5% bovine serum albumin (BSA; MilliporeSigma, St. Louis, MO) in 50 mM Tris buffer, pH 7.5 for one hour with gentle agitation. The prepared BSA blocked discs were frozen at -80°C and subsequently freeze-dried. These lyophilized BSA-blocked discs were used as a scaffold for the deposition of colorimetric wearable synthetic biology reactions in freeze-dried, cell-free (wFDCF) sensors. The reaction disks saturated with the cell-free reaction components were finally snap-frozen in liquid nitrogen and freeze-dried for 8 - 12 hours in an SP Scientific Freezemobile lyophilizer (SP Industries, Inc., Warminster, PA).

[00156] Freeze-dried reaction disks were then inserted through the wicking ports of the elastomer chambers for assembly. The silicone elastomer chambers in the colorimetric device exhibit three 3 x 5 mm curved wicking ports in each of the four reaction chambers, which allow routes for fluid entry while delaying evaporation of cell-free reaction (FIG. 7A). The device chamber walls were aligned and bonded using uncured elastomer, to prevent flow or lateral diffusion of the reaction after rehydration. The wicking of contaminated fluid through the entry ports is primarily mediated by capillary action. This event then leads to rehydration of the reaction disk containing the chosen FDCF system (FIG. 6A), which marks t = 0 in the validation experiments (FIGs. 6H-6K). A magnified photograph of an activated reaction well containing an Ebola virus DNA toehold wFDCF sensor is shown in FIG. 7B, whereas the activation of a fabricated wearable bracelet using the same system is depicted in FIG. 7C. All of the colorimetric wFDCF sensors were tested at 30°C and ambient humidity to simulate surface body temperature.

[00157] *Preparation of optimized colorimetric wearable synthetic biology reactions.*

[00158] Each colorimetric wFDCF reaction used for lyophilization, assuming a 50 µL rehydration volume, was a 75 µL cell-free NEB PUREXPRESS® reaction (New England Biolabs, Inc., Ipswich, MA). Thus, each rehydrated reaction is a 1.5x-concentrated cell-free

reaction based on the suggested reaction composition indicated by the manufacturer. Each reaction consisted of: 30 μL of PUREXPRESS® Component A, 22.5 μL of PUREXPRESS® Component B, 0.6 mg/mL of chlorophenol red-β-D-galactopyranoside (CPRG; MilliporeSigma, St. Louis, MO), 76 U of RNase Inhibitor (Roche GmbH, Mannheim, Germany), and a DNA template encoding the desired artificial genetic circuit at 5 ng/μL. For the TetR transcriptional regulation circuit, FPLC- purified recombinant TetR protein was supplemented in the reaction at a concentration of 120 μg/mL. During activation of the various wFDCF reactions by rehydration, pure nuclease-free H_2O was used for the constitutive LacZ circuit, 25 μg/mL of anhydrotetracycline (aTc) inducer was used for the TetR-regulated circuit, 300 nM of Ebola viral genome trigger was used for the toehold regulated circuit, and 1 mM of theophylline was used for the riboswitch-regulated circuit. The theophylline riboswitch reactions also included 2-Phenylethyl β-D-thiogalactoside (MilliporeSigma, St. Louis, MO), a β-galactosidase inhibitor, at a final concentration of 250 μM to suppress the background due to leakiness in these genetic circuits. The Ebola RNA genome trigger was acquired by an *in vitro* transcription reaction utilizing the HISCRIBE™ T7 Quick High Yield RNA Synthesis Kit (New England Biolabs, Ipswich, MA), using a DNA template. Each wFDCF reaction was applied to a BSA-blocked cellulose disc inserted into a 2 mL microcentrifuge tube. After the reaction was absorbed into the disc, the tubes were submerged in liquid nitrogen to snap freeze the disc and allowed to lyophilize for 12 hours. All of the colorimetric wFDCF sensors were tested at 30°C and ambient humidity to simulate surface body temperature. The colorimetric wFDCF presented in this work were from distinct sensors, in which each data point is the intensity value of a defined area of the green channel from the color-deconvolution function in ImageJ. The selected area size was kept constant for all sensors.

[00159] *Evaporation and dilution experiments in wearable synthetic biology devices.*

[00160] Evaporation tests were performed by cutting 10 x 10 cm WHATMAN™ No. 4 filter-paper squares and performing the cleaning and BSA blocking as described above for the discs. Each square was freeze-dried with 100 μL of a 1x PUREXPRESS® cell-free reaction with CPRG substrate and a constitutive LacZ plasmid. Various temperature (27-32°C) and fluid exposure conditions were investigated in combination with different coverage ratios of the rehydrated test squares to assess evaporation reduction. Suitable activity of the rehydrated reactions was assessed by visual inspection of the conversion of the colorimetric substrate from yellow to purple. The port designs shown in FIG. 7A, 8B, 17G were selected empirically due to suitable activation of synthetic biology reactions with reduced evaporation rates (<20% of initial fluid volume in 2 hours) at 30-40% relative humidity.

[00161] *Kinetic enhancement by freeze-dried concentration of cell-free reaction components.*

[00162] Optimization testing of cell-free component concentrations on the kinetics of the reactions was performed by assembling PUREXPRESS® systems, according to the manufacturer's specifications, at various volumes (Vinital) and then lyophilizing the reactions in PCR tubes overnight (FIG. 10A). Next, the lyophilized pellets were rehydrated using the same sample volume (Vfinal), so that the tested fold-concentration was (Vinital / Vfinal). PUREXPRESS® concentrations ranging from 1x to 2.5x were tested in replicate by incubation of 10 μL reactions at 30°C for up to 90 minutes, followed by photographic imaging of the colorimetric changes (FIG. 10B) and absorbance measurements at 570 nm (FIG. 10C). The time to half-maximal output signal for each base or concentrated reaction (FIG. 10D) was calculated by a least square fitting of the acquired data.

[00163] *Screening of textiles for freeze-dried cell-free synthetic biology reactions.*

[00164] General compatibility of different textiles to cell-free synthetic biology reactions was tested in 103 different fabrics materials (e.g., silks, cotton, rayon, linen, hemp bamboo, wool, polyester, polyamide, nylon, and combination threads) under activation conditions (FIG. 18A-18B). A detailed list of the textiles used for this substrate screening can be found in Table 1. This compatibility of these textiles to FDCF synthetic biology reactions was compared to samples using WHATMAN™ No. 4 filter paper (GE Healthcare Lifesciences Inc., Chicago, IL) and samples in liquid form without any substrate as seen in FIG. 19A-19B. All tests used a T7RNAP-regulated LacZ circuit for constitutive expression. For this evaluation, fabric samples were identified and cut into 2 x 2 cm squares. Visible particles were removed from the fabrics using an adhesive roller. All fabric squares were cut into 1 x 2 cm pairs and washed thoroughly within 1.5 mL Eppendorf tubes with 1mL dd-H_2O for 30 minutes floating in a sonication bath at 80°C. The washed samples were left to cool to room temperature and then washed with running dd-H2O for 10 sec. One of each pair of fabric square types was placed in 1.25 mL of a 5% BSA solution for 12 hours. After BSA incubation, the treated fabrics were cleaned with running dd-H_2O for 10 seconds. BSA-blocked and unblocked samples were then placed into fresh Eppendorf tubes with holes in the caps to allow for overnight desiccation of the fabrics at 60°C. Dried BSA blocked and unblocked fabrics were then cut in triplicate with clean 2 mm diameter disk biopsy punchers and placed in their respective slot in flat 384-well black polystyrene plates with a clear glass bottom (Corning Inc.; Kennebunk ME, Ref#. 3544) for testing. Cell-free PUREXPRESS® in vitro protein synthesis solution (New England Biolabs, Inc., Ipswich, MA) was combined with a constitutive LacZ template containing 0.6 mg/mL CPRG and spotted (1.8 μL) on each of the

fabric wells. Control wells containing 2 mm disks of Whatman No. 4 filter-paper were also filled with 1.8 μL constitutive LacZ test reactions, whereas 7 μL were spotted on empty wells as liquid controls. A transparent adhesive PCR cover compatible with freezing was then placed over the plate and pressed with a roller to seal chambers. A small opening was pierced in each well with a 25-gauge x 5/8 (0.5 mm x 16 mm) BD PrecisionGlide Needle (Becton, Dickinson and Company; Franklin Lakes, NJ, Ref#305122) to allow for sublimation during lyophilization. Prepared plates were wholly immersed into liquid nitrogen for 1 min. A chilled metallic plate (maintained at -80°C with dry ice), was immediately put in contact with the bottom of the scored plates with the sealed frozen samples. A single 15 x 17" Kimwipe (KIMTECH™, Kimberly-Clark Corp., Irving, TX) was placed on top of the plate humidity openings. Then the 384-well test plate with top Kimwipe and the bottom metallic chiller was wrapped with three layers of aluminum foil. The entire wrapped bundle was then placed inside a sealed glass lyophilization chamber and connected to the freeze-drying machine. Lyophilization was performed for two hours. Freeze-dried paper samples were rehydrated with dd-H2O to the original reaction volume. The colorimetric change was measured after overnight incubation (12 hours) at 37°C using a BioTek NEO HTS plate reader (BioTek Instruments, Inc., Winooski, VT) in kinetic absorbance readout mode FIG. 19A-19B. Best observed functionality, as measured by the aggregated score shown in FIG. 20, was achieved using a fabric with 85% polyester and 15% polyamide fibers. This substrate was used for all further fluorescence and luminescence experiments, except for the case for a fluorescence Zika DNA Toehold sensing reaction (FIG. 11), which was also tested on a 100% mercerized cotton thread to validate the possibility of running FD-CF reactions at the single fiber level with this natural material commonly used in wound care.

[00165] *Fabrication of fluorescence/luminescence synthetic biology wearable textile module*

[00166] After screening of compatible textiles for freeze-dried, cell-free synthetic biology reactions, the best performing hydrophilic textile substrate (85% polyester / 15% polyamide) was used as weft for a textile inter-woven with a warp made of inert flexible polymeric optic fibers (POF) and polyester support threads. Such POFs were used for distributed optical interrogation of fluorescent or luminescent synthetic biology reactions within this fabric (three fibers per well). Polymeric optic fibers were weaved into this hydrophilic combination fabric using a standard industrial loom (DREAMLUX, Samsara Srl., Milan, IT), according to the design presented in FIGs. 16A-16D. Once fabric samples were manufactured, three-strip arrangements of this hydrophilic POF fabric were cut to fit the device and laser-etched (5 mm) to disrupt the cladding in the POFs sections within the reaction zones (FIGs. 17A-17G). Black elastomer layers (top and bottom in FIG. 17B) were precast overnight and laser-cut according to the layout

shown in FIGs. 17B, 17E. The silicone elastomer chambers in this device exhibit two 3 x 5 mm curved wicking ports that allow for fluid entry while still delaying evaporation within reaction fabric. Uncured black silicone elastomer was stamp-patterned onto the precast layers as well as into the internal POF fabric strips to be aligned and assembled, preventing air bubble formation between device layers and elastomer wicking in reaction zones. Final assembly of the base three-well sensor "patch" can be seen in FIGs. 17B, 17F, 17G. Devices were then placed under vacuum for 15 minutes to remove bubbles and were allowed to cure overnight at 65°C. As with the colorimetric prototypes, the fluorescent POF prototypes were thoroughly sprayed with RNase Away Decontaminant (Thermo Fisher Scientific, Waltham, MA) and washed with 70% ethanol twice before being stored in petri dishes. Once the assembled device was fully cured, POF fibers were separated into excitation and emission bundles and then covered with blackout adhesive fabric as well as black heat shrink tubing (6 mm) to prevent environmental light leakage. Blackout fabric disks (10 mm) made of black polyester knit Item#: 322323 (MoodFabrics Inc. New York, NY) were soaked in RNase Away Decontaminant for 5 minutes, washed thoroughly with 70% ethanol followed by water. The washed blackout fabric was incubated in 0.1% Triton X-100 for 5 minutes (as a wetting agent to enhance the ability of the textile to absorb water) and then excess solution was removed and the fabric pieces allowed to air-dry. The final blackout fabric discs were placed inside the reaction chamber with tweezers to aid in environmental light-blocking over sensing fibers. Finally, quick-turn stainless steel coupling sockets #5194K42 (McMaster-Carr Co., Elmhurst, II) were added to the ends of the sensor device bundles for connection with the wearable spectrometer. The finalized wFDCF sensor device can be seen in FIGs. 17F, 17G.

[00167] *Hardware / software implementation of wearable POF spectrometer*

[00168] A custom-made wearable spectrometer with internal processing and wireless connectivity modules was fabricated to provide unsupervised sensing of on-body synthetic biology reactions (FIGs. 21A-21F). The device electronics were based on a Raspberry Pi Zero W Version 1.3 architecture (Raspberry Pi Foundation, Cambridge, UK) with connection to a custom shield for battery power, an environmental sensing module, an LED illumination module, and a flexible camera for imaging (FIG. 21A). The Raspberry Pi Zero W was selected as microprocessing for this application, due to its low cost (<$15.00), small profile/weight (65 x 30 x 5 mm / 12 g), high performance (1 GHz single-core ARM1176JZF-S CPU, 512 MB RAM, VideoCore IV GPU) and on-board wireless connectivity (802.11 b/g/n LAN, Bluetooth(R) 4.1, Bluetooth Low Energy -BLE). Regulated battery power was achieved using a PiZ-UpTime module, which is an uninterruptible power supply shield for Raspberry Pi Zero (Alchemy Power

Inc., Santa Clara, CA), which uses rechargeable a Lithium-Ion 14500 battery (Battery & Power management in FIG. 21A), to reliably provide the charge capacity for 48 hrs of intermittent device operation continuously collecting data at a frequency of one measurement per minute. In-device sensing of temperature, humidity, atmospheric pressure, altitude, total Volatile Organic Compound (TVOC) and eCO2 was achieved using an I2C environmental CCS811/BME280 Qwiic-Breakout (SPARKFUN ELECTRONICS®, Niwot, CO). The POF illumination module was achieved using a Saber Z4 Luxeon Z 20 mm Square Quad Color Mixing Array LED Module with aluminum base (Quadica Developments Inc. - Luxeon, Alberta, Canada) connected to a 12-Channel 16-bit PWM TLC59711 LED driver with SPI Interface (ADAFRUIT INDUSTRIES®, New York, NY). Four Luxeon Star LEDs were installed in the device with wavelengths 447 nm, 470 nm, 505 nm and 6500 K white (LEDs & Driver in FIG. 21A). An 8.6 mm x 8.6 mm Zero Spy Camera with 2" cable (Raspberry Pi Foundation, Cambridge, UK) was connected to the Raspberry Pi Zero W using a

flat serial interphase connector to provide POF imaging capabilities to the device. A single 5 mm INFINITE© aspherical plastic collimator part#: 191-66041G (Quarton Inc., New Taipei City, Taiwan) with numerical aperture (NA): 0.27 and effective focal length (EFL): 4.96 mm, was placed on top of the camera to allow for magnified POF imaging in proximity to the camera. The wearable spectrometer was covered by a two-part case fabricated using black photoreactive resin and a stereolithography 3D printing method using a Form 2 printer (Formlabs Inc., Summerville, MA) as seen in FIG. 21A. A view of the open device is shown in FIG. 21B, while a closed view is shown in FIG. 21C. This case included geometrical features to fit and align the camera/lens arrangement and the removable 3 mm diameter amber acrylic filter for fluorescence readings (slot arrangement in FIG. 21D). Also, the case features a slot for the 4-LED arrangement, a vent for the environmental sensors (FID. 21D), as well as female Luer connection (FIG. 21A) to fit quick-turn stainless steel coupling sockets #5194K42 (McMaster-Carr Co., Elmhurst, II). A top view of the assembled wearable POF spectrometer is shown in FIG. 21E, while the integration of this device within a wearable garment with wFDCF sensors is shown in FIG. 21F. The final volume of the wearable spectrometer device was approximately 235 cm^3 with a total weight of around 173.8 grams (6.13 ounces), with a total cost of material and consumable supplies under $100 USD. Base data-collection software (test version) implemented in python for control of the Raspberry Pi Zero W within the wearable POF spectrometer was also provided.

[00169] *Preparation of optimized fluorescence wearable synthetic biology reactions.*

[00170] Constitutive sfGFP expression reactions for wFDCF testing (FIG. 9C) were prepared by combining 50 µL of 1x NEB cell-free PURExpress® in vitro protein synthesis solution with 0.5% Roche Protector RNase Inhibitor and 10 ng/µL constitutive PT7-sfGFP plasmid (+) or without as controls (-). Prepared reactions were quickly deposited in-fabric to be snap-frozen and then lyophilized for 4-8 hours within the device. Activation of sensors was achieved by rehydration with a fluid splash of dd-H_2O.

[00171] Theophylline riboswitch sensor reactions for wFDCF testing (FIG. 9D) were prepared using 1x NEB cell-free PURExpress® with 10 ng/µL Theophylline riboswitch sensor E mRNA in dd-H_2O prepared sensor reactions (50 µL per well) were quickly deposited in-fabric to be snap-frozen and then lyophilized for 4-8 hours within the device. Activation of sensors was achieved by rehydration with a fluid splash of dd-H_2O spiked with 1 mM theophylline for the positive samples, while 0 mM theophylline was used for controls.

[00172] Dimeric Broccoli fluorescent aptamer sensor reactions for wFDCF testing (FIG. 9E) were prepared using 1.5x NEB cell-free PURExpress® with 25 ng/µL of pJL1-F30-2xd-Broccoli aptamer DNA in dd-H_2O. Prepared sensor reactions (50 µL per well) were quickly deposited in-fabric to be snap-frozen and then lyophilized for 4-8 hours within the device. Activation of sensors was achieved by rehydration with a fluid splash of dd-H_2O spiked with 50 µM of the substrate (5Z)-5-((3,5-Difluoro-4-hydroxyphenyl)methylene)-3,5-dihydro-2-methyl-3-(2,2,2-trifluoroethyl)-4H-imidazol-4-one (DFHBI-1T; Tocris Bioscience, Minneapolis, MN) substrate for the positive samples, while 0 µM DFHBI-1T substrate was used for controls.

[00173] Zika RNA Toehold switch sensor reactions for wFDCF testing (FIG. 11) were prepared using 1x NEB cell-free PURExpress® with 33 nM Zika DNA toehold sensor 27B in dd-H_2O. Prepared sensor reactions were quickly deposited in a mercerized cotton thread or paper samples to be snap-frozen and then lyophilized for 4-8 hours within a 384-well plate. Activation of sensors was achieved by rehydration with dd-H_2O spiked with 2 µM of freshly made Zika trigger RNA for the positive samples, while 0 µM Zika trigger RNA was used for controls.

[00174] For the wearable nerve agent sensor experiments (FIG. 9G), 50 µL reactions consisting of 0.5 U/mL acetylcholinesterase (Type V-S from *E. electricus*, MilliporeSigma, St. Louis, MO), 0.1 U/mL of choline oxidase (recombinant *Arthrobacter sp.*, MilliporeSigma, St. Louis, MO), 0.1 mg/mL of freshly prepared horseradish peroxidase (Type VI, MilliporeSigma, St. Louis, MO), and 125 µM of the fluorescent reporter substrate AMPLITE-IR™ (AAT Bioquest, Sunnyvale, CA) in a final buffer of 10 mM HEPES, pH 8.0 / 1 mg/mL BSA / 1% fish gelatin / 5% trehalose. The reactions were applied to two WHATMAN™ No. 4 filter-paper 0.8

cm discs, snap frozen in liquid nitrogen, and lyophilized for at least 12 hours. To test in the fluorescent wearable prototype, the paper discs containing the freeze-dried reactions were inserted into the wearable devices and rehydrated with 75 μL of 50 μM acetylcholine (MilliporeSigma, St. Louis, MO) with or without the nerve agent paraoxon-ethyl (MilliporeSigma, St. Louis, MO). The fluorescent wearable device for the nerve agent was altered for the detection of near-infrared fluorescence by replacing the optical components with excitation using a 627 nm red quad-LED array module (Quadica Developments Inc. - Luxeon, Alberta, Canada). Additionally, the emission camera was substituted with a NoIR Zero Spy Camera without infrared filter, on top of which was positioned three gel transmission filters No. 381, 382 and 383 (Rosco Laboratories Inc., Stamford, CT) to form a dedicated emission filtering stack with <1% cutoff at 660nm and peak transmittance at 740nm. All of the fluorescent wFDCF sensors were tested at 30°C and ambient humidity to simulate surface body temperature. All fluorescent wFDCF presented in this work were from distinct sensors, in which each data point is the integrated value of optical fiber signals from one sensor. Any fiber optic fibers that were 1 SD below the mean of all fibers combined were removed from analysis.

[00175] *Preparation of optimized luminescence wearable synthetic biology reactions.*

[00176] HIV RNA toehold switch sensor reactions for luminescence wFDCF testing (FIG. 9F, 15B) were prepared in 50 μL batches using 20 μL of NEB cell-free PUREXPRESS® Component A, 15 μL NEB Component B, 2.5 μL murine RNase inhibitor (New England Biolabs, Inc., Ipswich, MA), 6 ng/μL HIV toehold sensor template with a nano luciferase (nLuc) output, 0.5 μL luciferin substrate (Promega Corp., Madison, WI) in dd-H_2O. Prepared sensor reactions (50 μL per well) were quickly deposited in-fabric to be snap-frozen and then lyophilized for 4-8 hours within the device. Activation of sensors was achieved by rehydration with a fluid splash of dd-H_2O spiked with 10 μM HIV trigger RNA freshly made for the positive samples, while 0 μM HIV trigger RNA was used for controls. The constitutive nLuc control reaction shown as part of FIG. 15B was performed similarly but substituting the toehold switch with a plasmid with a nLuc operon regulated by a T7 promoter.

[00177] *Borrelia burgdorferi* RNA Lyme disease toehold switch sensor reactions for luminescence wFDCF testing (FIG. 15A) were prepared in 50 μL batches using 20 μL of NEB cell-free PUREXPRESS® solA, 15 μL NEB solB, 2.5 μL murine RNAse inhibitor, 18 nM *B. burgdorferi* toehold DNA with luciferase operon, 2.75 μL luciferin substrate (Promega Corp., Madison, WI) in dd-H_2O. Prepared sensor reactions (50 μL per well) were quickly deposited in-fabric to be snap-frozen and then lyophilized for 4-8 hours within the device. Activation of

sensors was achieved by rehydration with a fluid splash of dd-H2O spiked with 3 μM *B. burgdorferi* trigger RNA freshly made for the positive samples, while 0 μM trigger RNA was used for controls. These wFDCF sensors were tested at 30°C and ambient humidity to simulate surface body temperature.

[00178] *Preparation of optimized CRISPR Cas12a-based wearable synthetic biology reactions.*

[00179] CRISPR-based sensor reactions for wFDCF testing in FIG. 23B-23F were prepared using 100 nM Cas12a (New England Biolabs, Ipswich, MA) and 100 nM gRNA, 1x NEB buffer 2.1, 0.45 mM dNTPs, 500 nM of each RPA primer, 1x RPA liquid basic mix (TwistDx Limited, UK), 14 mM $MgCl_2$, and 5 μM FAM-IOWA BLACK® FQ quenched ssDNA fluorescent reporter (Integrated DNA Technologies, Coralville, IA) in dd-H2O. Prepared sensor reactions (50 μL per well) were quickly deposited in-fabric to be snap-frozen and then lyophilized for 4-8 hours within the device. Activation of sensors was achieved by rehydration with a fluid splash of dd-H_2O spiked with 2.7 fM or 100 fM of *mecA*, *spa* or *ermA* DNA trigger depending on the demonstration. In the sensing performed at 2.7 fM *mecA* trigger, the detection limit is 10,000 copies of DNA per μL. These wFDCF sensors were tested at 30°C and ambient humidity to simulate surface body temperature.

[00180] *Preparation of optimized CRISPR Cas13a-based wearable synthetic biology reactions.*

[00181] Cas13a CRISPR-based sensor reactions for wFDCF testing (FIG. 25) were prepared using 100 nM Cas13a and 100 nM gRNA, 1x NEB buffer 2.1, 0.45 mM dNTP, 14 mM $MgCl_2$, and 5 μM FAM-IOWA BLACK® FQ quenched RNA fluorescent reporter (Integrated DNA Technologies, Coralville, IA) in dd-H_2O. Prepared sensor reactions (50 μL per well) were quickly deposited in-fabric to be snap-frozen and then lyophilized for 4-8 hours within the device. Activation of sensors was achieved by rehydration with a fluid splash of dd-H_2O spiked with 20 nM of MRSA RNA trigger.

[00182] *Preparation of sample lysis-integrated wearable synthetic biology reactions.*

[00183] For wFDCF with integrated lysis reactions, a RNase-free Whatman filter paper disc (8 mm) was filled with concentrated stock solutions that would yield, upon a 50 μL rehydration volume, 5 mM Tris-HCl (pH 7.5), 1% Triton X-100, 1% NP-40, 0.2% CHAPS, 100 μg/mL lysozyme, and 5% sucrose. This was freeze-dried for 4 hours and inserted into the POF wFDCF device below the blackout layer and above a PVA time delay barrier that was sealed around the edges with EcoFlex elastomer to enable an efficient lysis incubation time. All layers containing

the lyophilized RPA-Cas12a synthetic biology sensors below the lysis – PVA delay layers were identical to that used in the *mecA* RPA-Cas12a devices shown in FIG. 23B-23E.

[00184] *Garment-level integration of colorimetric synthetic biology sensors.*

[00185] After fabrication of colorimetric synthetic biology wearable module, a bracelet "garment" was achieved simply by gluing the module into an elastic band to be placed in the forearm of a mannequin (FIG. 8C).

[00186] *Garment-level integration of fluorescence/luminescence synthetic biology sensors.*

[00187] After fabrication of at least 12 fluorescence/luminescence synthetic biology wearable modules, a commercially available long-sleeve neoprene wetsuit-type jacket (Eyce Dive & Sail) was modified to integrate an array of wFDCF sensors by sewing these modules in predefined high- splash frequency regions (FIG. 9A, 21F). Reaction modules were covered in the edges with a blackout fabric border with textile adhesive. POF bundles of these modules were sawn internally and directed to a single multi-bundle arrangement for interrogation via the portable spectrometer device (located in a back pocket within the jacket) as seen in FIG. 21F. Base neoprene fabric used for this jacket was of 3mm thickness and treated with a superhydrophobic coating to prevent fluid absorption in places other than the reaction zones. Fabricated wFDCF jacket prototype was specified to fit a medium-sized male torso 36"(chest) by 31"(waist). In-garment sensors were tested on a mannequin at room temperature.

[00188] *Sensor and reporter sequences*

Tables S2 and S3 contain the DNA and RNA sequences of sensors and reporters used in this study. The plasmid construct used for the Zika 27B toehold sensor has been previously described elsewhere. The Lyme disease and HIV toehold sensors with a nanoluciferase output were cloned into the pBW121 plasmid backbone (Addgene plasmid #68779). All other plasmid constructs utilized the pJL1 backbone that has been previously described2, [3]. The F30 dimeric Broccoli fluorescent aptamer was subcloned into pJL1 from pET28c-F30-2xdBroccoli which was a gift from Samie Jaffrey (Addgene plasmid #66843; www.n2t.net/addgene:66843; RRID:Addgene_66843). The sequence for the pJL1-sfGFP plasmid can be found on Addgene (Plasmid #69496).

TABLE 1: Fabrics tested – results shown in FIG. 20

ID	Supplier	Supp. ID	Material	Type	Name	Manufacturer Description
0	-	N/A	-	No substrate (control)	No substrate (control)	-
1	Whatman GE	1442-042	Cellulose Paper	42.5mm filter papers	42.5mm filter papers	Ashless filter papers with 0.2mm thickness and 42.5mm diameter.
2	Dharma Trading Co. (DTC)	SBC45	Silk	Spun Silk Broadcloth 45" wide	Spun Silk Broadcloth 45" wide	100% spun silk 23mm, mid-weight flat woven fabric with a crisp hand and slight sheen, drapes nicely.
3	DTC	SD	Silk	Silk Dupion 19mm 45" wide	Silk Dupion 19mm 45" wide	Picture shimmery silk with lots of texture - raised silk slubs - intermittent shiny threads - similar to Shantung. Makes outstanding jackets, suits, coats, etc.
4	DTC	SG36	Silk	Silk Gauze 3mm	Silk Gauze 3mm 36" wide	Wide weave. Very light, very sheer, very beautiful.
		SG45	Silk		Silk Gauze 3mm 45" wide	Wide weave. Very light, very sheer, very beautiful.
		SG55	Silk		Silk Gauze 3mm 55" wide	Wide weave. Very light, very sheer, very beautiful.
5	DTC	SVEL45	Silk	Silk Velvet	Silk Velvet 45" wide	Standard silk, 82% rayon. Dyes and paints super. The ultimate! Use for the Devore techmque.
		SVEL55	Silk		Silk Velvet 55" wide	Standard silk, 82% rayon. Dyes and paints super. The ultimate! Use for the Devore techmque.
6	DTC	STCHARM	Silk / Spandex	Stretch Charmeuse 12mm 45" wide	Stretch Charmeuse 12mm 45" wide	All die softness and sheen of silk with the comfort of a Stretch fabric. 95% silk & 5% spandex. The low amount of spandex will not the dyeing process.
7	DTC	ST	Silk	Silk Twill 12mm 45" wide	Silk Twill 12mm 45" wide	A very utilitarian durable silk. Great for shirts, handkerchiefs, pillow cases, lamp shades, screens, etc.
8	DTC	SRS45	Silk	Smooth Raw	Smooth Raw Silk 31.5mm	100% Silk 45" width

				Silk 31.5mm		
9	DTC	SG4545	Silk	Silk Gauze 4.5MM 45" wide	Silk Gauze 4.5MM 45" wide	Some like it a bit heavier. Beautiful flowing fabric, great for sewing and gifts.
10	DTC	SK45	Silk	Silk Knit 45" wide	Silk Knit 45" wide	100% Silk Jersey, 3oz/sq.yd approx. 100 g/m2, 22" wide tube (44" wide), soft hand with a nice drape, great for silk underwear garments and scarves.
11	DTC	ORG45	Silk	Organza 5.5 mm	Organza 5.5 mm 45" wide	Very sheer, transparent, stiff, gauze-like fabric.
		ORG55	Silk		Organza 5.5 mm 55" wide	Very sheer, transparent, stiff, gauze-like fabric.
12	DTC	CBSS36	Silk	Crepe Back Silk Satin (Charmeuse)	Crepe Back Silk Satin (Charmeuse) 19.5mm 36" wide	It has a firm feel to the hand and a luxuriously heavy drape. Dyes and paints will show an intensity of color and richness on this fiber.
		CBSS45	Silk		Crepe Back Silk Satin (Charmeuse) 19.5mm 45" wide	It has a firm feel to the hand and a luxuriously heavy drape. Dyes and paints will show an intensity of color and richness on this fiber.
		CBSS55	Silk		Crepe Back Silk Satin (Charmeuse) 19.5mm 55" wide	It has a firm feel to the hand and a luxuriously heavy drape. Dyes and paints will show an intensity of color and richness on this fiber.
13	DTC	VRS	Rayon / Silk	Vicose Rayon / Silk Blend	Vicose Rayon / Silk Blend	75% Rayon / 25% Silk blend. Weight equivalent to 18mm. Although fabric is semi-sheer when white. when dyed it is mostly opaque. 58"wide
14	DTC	TAF55	Silk	Silk Taffeta - 14.5mm	Silk Taffeta -14.5mm	100% Silk 55" width
15	DTC	SWSC	Silk	Sand Washed (Sueded) Charmeuse 19.5mm 15" w	Sand Washed (Sueded) Charmeuse 19.5mm 15" wide	Beautiful & desirable! people here are taking up sewing because of it.
16	DTC	SWCDC	Silk	Stone wash Crepe de Chine	Stone wash Crepe de Chine	By stone (sand) washing this heavier weight of Crepe de Chine, one gets a matte silk with a soft, luxurious drape. This is a great weight for any almost article of clothing items like pillows, curtains and such.
17	DTC	SWF	Silk / Wool	Silk / Wool blend 63% silk, 37% wool	Silk / Wool blend 63% silk, 37% wool	Slightly sheer with a diagonal weave almost like a chiffon but sturdier. This material is softer than wool warmer than silk 12.5mm 55" wide.

18	DTC	CHIF16	Silk	Silk Double Chiffon 16mm 45"	Silk Double Chiffon 16mm 45"	With a crepey texture and a low subtle sheen, this is a perfect weight and drape for blouses. dressy slacks, or skirts. Takes dye beautifully
19	DTC	CHIF10	Silk	Silk Chiffon 10 mm, 54"	Silk Chiffon 10 mm, 54"	An elegant, sheer fiber with a soft beautiful drape and a crepe like texture. Heavier and wider than the 8mm.
20	DTC	CHIF44	Silk	Silk Chiffon 8mm	Silk Chiffon 8mm 44"	An elegant. sheer fiber with a soft drape and a crepe like texture.
		CHIF55	Silk		Silk Chiffon 8mm 55"	An elegant. sheer fiber with a soft drape and a crepe like texture.
21	DTC	CDC1236	Silk	Crepe de Chine	Crepe de Chine 12mm 36" wide	Soft luxurious crepe texture with great draping quality. Comes in 36"/45"/55" Width
		CDC1245	Silk		Crepe de Chine 12mm 45" wide	Soft luxurious crepe texture with great draping quality. Comes in 36"/45"/55" Width
		CDC1255	Silk		Crepe de Chine 12mm 55" wide	Soft luxurious crepe texture with great draping quality. Comes in 36"/45"/55" Width
22	DTC	CDC45	Silk	Crepe de Chine	Crepe de Chine 16mm 45"	Slightly crinkled texture with a gentle, graceful drape and very soft hand. Has a smooth surface that lends itself well to all types of painting styles. Ideal for all types of clothing and decorating.
		CDC55	Silk		Crepe de Chine 16mm 55"	Slightly crinkled texture with a gentle, graceful drape and very soft hand. Has a smooth surface that lends itself well to all types of painting styles. Ideal for all types of clothing and decorating.
23	DTC	CCDC45	Silk	Crepe de Chine	Crepe de Chine	100% Silk, Optically Whitened — 16mm, 45" width
24	DTC	CBSSL36	Silk	Crepe Back Silk Satin '	Crepe Back Silk Satin 36"	100% Silk 12mm lightweight satin with a nice sheen Dn one side, matte texture on the other side, drapes nicely and feels fabulous.
		CBSSL45	Silk		Crepe Back Silk Satin 45'"	100% Silk 12mm lightweight satin with a nice sheen Dn one side, matte texture on the other side, drapes nicely and feels fabulous.
		CBSSL55	Silk		Crepe Back Silk Satin 55"	100% Silk 12mm lightweight satin with a nice sheen Dn one side, matte texture on the other side,

						drapes nicely and feels fabulous.
25	DTC	HS16	Silk	Silk Habotai	Silk Habotai 16mm 45" wide	The basic "China Silk" but even heavier
26	DTC	HCJ	Hemp / Cotton	Hemp/Cotton Jersey	Hemp/Cotton Jersey	55% Hemp, 45% Cotton — 50z, 61" width
27	DTC	HS1036	Silk	Habotai Silk lomm	Habotai Silk lomm 36"-103"	Heavier weight. Great for pillows, jackets, banners.
		HS1045	Silk		Habotai Silk lomm 36"-103"	Heavier weight. Great for pillows, jackets, banners.
		HS1055	Silk		Habotai Silk lomm 36"-103"	Heavier weight. Great for pillows, jackets, banners.
		HS1070	Silk		Habotai Silk lomm 36"-103"	Heavier weight. Great for pillows, jackets, banners.
28	DTC	HS12	Silk	Silk Habotai	Silk Habotai 12mm 45" wide	The basic China Silk, but heavier
29	DTC	HS836	Silk	Habotai Silk	Habotai Silk 8mm 36" wide	When they say China Silk this is What they mean. Light Weight and sheer. The 8mm is the one most seen, and of which the Habotai scarves are made. Great for pillows, linings for jackets, etc.
		HS845	Silk		Habotai Silk 8mm 45" wide	When they say China Silk this is What they mean. Light Weight and sheer. The 8mm is the one most seen, and of which the Habotai scarves are made. Great for pillows, linings for jackets, etc.
		HS855	Silk		Habotai Silk 8mm 55" wide	When they say China Silk this is What they mean. Light Weight and sheer. The 8mm is the one most seen, and of which the Habotai scarves are made. Great for pillows, linings for jackets, etc.
30	DTC	HS536	Silk	Habotai Silk	Habotai Silk 5mm 36" wide	When they say China Silk this is What they mean. Light Weight and sheer.Everyone likes this stuff.
		HS545	Silk		Habotai Silk 5mm 45" wide	When they say China Silk this is What they mean. Light Weight and sheer.Everyone likes this stuff.
		HS555	Silk		Habotai Silk 5mm 55" wide	When they say China Silk this is What they mean. Light Weight and sheer.Everyone likes this stuff.
31	DTC	FCR4S	Silk	Flat Crepe	Flat Crepe 8mm 45" Wide	A fibric that looks like a combination of the texture

						and drape of crepe and luster of habotai. It is easy to dye and paint and accepts readily all types of painting techniques.
32	DTC	DSAT45	Rayon / Silk	Devore Satin (30% Silk / 70% Rayon)	Devore Satin (30% Silk / 70% Rayon)	19 mm 45" wide. Silky, shiny beautiful fabric for "burn-out" (apply "Fiber-Etch" to remove the Rayon threads in a design leaving a lace of silk)
33	DTC	HCDC	Silk	Heavy Crepe de Chine	Heavy Crepe de Chine 30mm 45"	Like the 16mm crepe de chine, only heavy enough to take dye with great depth and intensity. With a dull sheen and a soft dape it is perfect for pants, jackets, etc.
34	DTC	JAC9702	Silk	Inkjet Printable Silk fabric	Inkjet Printable Silk fabric	Inkjet silk fabric sheets 1.5"x11" comes with detachable paper support
35	DTC	JAC9703	Silk	Semi-transparent Inkjet Printable Silk fabric	Semi-transparent Inkjet Printable Silk fabric	Thin and semi-transparent inkjet silk fabric sheets 1.5"x11" comes with detachable paper support
36	DTC	PWFC	Wool	Pure Wool Gauze Twill Fabric	Pure Wool Gauze Twill Fabric 45"	100% wool, lightweight with a subtle twill weave, 45" wide, 3.2 oz / linear yard
37	DTC	PWFA	Wool	Pure Wool Gauze Fabric	Pure Wool Gauze Fabric 45"	100% wool, lightweight and semi-sheer, 45" wide, 3.2 oz / linear yard
38	DTC	PHC	Hemp	Hemp Canvas	Hemp Canvas	100% Hemp - 11 oz, 53" width, optically whitened
39	DTC	HWM	Hemp	Hemp Woven Mid-weight	Hemp Woven Mid-weight	100% Hemp - 5.4 oz, 53" width, optically whitened
40	DTC	HSCH	Hemp / Silk	Hemp Silk Charmeuse	Hemp Silk Charmeuse	60% hemp, 40% silk. Silk lends a beautiful shine to one side, while the hemp lends strength to the body. 57" wide
41	DTC	HS	Hemp / Silk	60% Hemp / 40% Silk	60% Hemp / 40% Silk	Natural. 57" wide. A spectacular fabric. Beautiful, soft and interesting. If this fabric was human you would want to marry it. The elegance of silk and the strength of hemp. Vertical textured grain., 2.6 oz/yd
42	DTC	HR	Hemp / Rayon	55% Hemp / 45% Rayon	55% Hemp / 45% Rayon	Natural, 59" wide. Soft and smooth, great for dresses and other clothing items. It washes well. 5.5 oz/yd
43	DTC	HM	Hemp	Hemp Summer	Hemp Summer Cloth	100% Hemp - 70z, 53" width. Optically whitened

				Cloth		
44	DTC	HCM	Hemp / Cotton	Hemp Cotton Muslin	Hemp Cotton Muslin	55% Hemp, 45% Cotton - 4.15z. 53" width. Optically whitened
45	DTC	PTC45/58	Cotton	Pimatex Cotton - PFD	Pimatex Cotton - PFD	High quality, great for sewing fine clothing. For that special occasion blouse, dress shirt or heirloom item. Dyes as well or better than mercerized cotton 133x72 thread count, 45" wide, 3.7 oz/yd.
46	DTC	POP60	Cotton	Poplin	Poplin	100% Cotton tightly woven, medium weight fabric 6 oz pet yard Wide. Thread count is 108 threads per inch x 50 threads per inch.
47	DTC	WJ242	Rayon	Rayon Satin, Heavyweight	Rayon Satin, Heavyweight 58"	Satin refers to a particular type of weave which results in a fabric with a very solid, tightly woven look. Optically whitened. 58" wide.
48	DTC	VRL	Rayon	Vicose Rayon Light	Vicose Rayon Light	100% rayon, Optically whitened, 4 oz per sq yd, 45" wide threads per inch x 38 threads per inch.
49	DTC	VCI	Rayon	Viscose (Rayon) Challis (Import)	Viscose (Rayon) Challis (Import)	Often seen in hawaiian shirts and ladies' wrap skirts. Because rayon is pure cellulose, it allows dyes to give very brilliant colors. 58" wide, 4 oz/yd, 90x60 threads.
50	DTC	SCVO	Cotton	Silky Cotton Voile	Silky Cotton Voile	Similar to the Cotton Voile, but with a tighter weave and a nice sheen this fabric has a very smooth silky 1.9 oz/yd sq. 52" wide
51	DTC	SCN	Cotton	Slubby Cotton Knit	Slubby Cotton Knit	100% cotton - 30z. 58"width
52	DTC	BBF	Bamboo Rayon	Bamboo Rayon	Bamboo Rayon	100% bamboo rayon. It's beautiful, soft and luxurious. 3.2 oz /sq yd, 60" wide. Thread count is 76x76 threads per inch.
53	DTC	BAMF	Bamboo Rayon	100% Bamboo Rayon Fleece	100% Bamboo Rayon Fleece	This fabric is really soft. One side is a smooth knit while the flip side is a soft fleece. 14oz per square yd, 60" wide.
54	DTC	SOCJ	Soy Cotton	Soy Organic Cotton Jersey	Soy Organic Cotton Jersey	58% Soy, 37% Organic cotton, 5% Spandex. This is a lovely and extremely soft jersey with a wonderful drape. 6.3oz per square yd, 60"wide.
55	DTC	RSAT45	Rayon	Rayon Satin	Rayon Satin	Satin refers to a particular type of weave which

						results in a fabric with a very solid, tightly woven look. 45" wide. Optically Whitened.
56	DTC	RV045	Rayon	Rayon Voile	Rayon Voile	Gorgeous sheen and a soft smooth hand. Weave is loose, somewhat sheer, and drapes well. 45" wide Optically Whitened.
57	DTC	RT55	Rayon	Rayon Twill	Rayon Twill	Has perhaps the nicest drape of any of the fabrics, including silks. Strong twill weave that will last. 55" Wide. Optically Whitened.
58	DTC	RL55	Rayon	Rayon Lawn	Rayon Lawn	100% rayon very smooth and has a fine weave. 2.5 oz per square yard, 54/55" wide. Thread count is 90 threads per inch x 88 threads per inch.
59	DTC	RCK	Rayon	Rayon Crinkle	Rayon Crinkle	beautiful, light crinkled rayon - great fabric for all kinds of clothing. 86 x 44, 4.5 oz. / sq. yd. approx.
60	DTC	QCS	Cotton	Cotton Sateen	Cotton Sateen	Sateen has a smooth finish on one side, kind of a sheen. This softer, finely woven fabric is used for lots more than quilts.
61	DTC	JER40	Cotton	Jersey Cotton	Jersey Cotton	100% Cotton slightly stretchy fabric with a smooth flat face. It's what basic t-shirts are made of. 6.50z per linear yd 58/60" wide.
62	DTC	MUS5B	Cotton	Premier Muslin Bleached	Premier Muslin 60" Bleached	Bleached midweight muslin. 100% Cotton, 3.2 oz per square yard, 60" wide, Thread count is 68 threads per inch x 68 threads per inch
63	DTC	NF	Nylon	Nylon	Nylon 58" wide	Untreated dye able nylon. Prepared for printing. Perfect tor banners or flags. Stronger outdoors than natural fibers.
64	DTC	NET48	Cotton	Cotton Net Fabric	Cotton Net Fabric 48"	100% cotton, slight stiffness disappears when washed, 33yd bolt comes pre-folded in a bag, 48" wide.
65	DTC	NPF	Nylon	Nylon 'Puppet Skin' Fleece	Nylon 'Puppet Skin' Fleece	100% Nylon - 120z, 54"/56" width.
66	DTC	NVEL	Rayon / Nylon	Rayon/Nylon Velvet	Rayon/Nylon Velvet	80% Rayon, 20% Nylon - 4.20z, 45" width.
67	DTC	OCDT	Cotton	Organic Cotton Denim Twill	Organic Cotton Denim Twill	This denim is strong and versatile. Anywhere you might use denim or twill. 70z per square yd, 56"

						wide.
68	DTC	MJ60	Rayon	Modal Rayon Jersey	Modal Rayon Jersey	95% Rayon, Lycra. Rayon jersey is extremely popular in ready to wear at all the best retail stores. 100z / linear yard. 60" wide.
69	DTC	MCCB45	Cotton	Mercerized Combed Cotton Broadcloth	Mercerized Combed Cotton Broadcloth 45" wide	Very finely Woven, 133 x 72 threads per inch. Mercerized to take dyes and paints better. 60" Wide. For batik and fine painting. 3.5 oz/yd sq.
		MCCB60	Cotton		Mercerized Combed Cotton Broadcloth 60" wide	Very finely Woven, 133 x 72 threads per inch. Mercerized to take dyes and paints better. 60" Wide. For batik and fine painting. 3.5 oz/yd sq.
70	DTC	LR	Linen / Rayon	55% Linen / 45% Rayon	55% Linen / 45% Rayon	Want the soft drape of rayon and cool comfort of linen? This is the one fabric for you. The linen look is heavy enough for pant, drapey enough for tops and dresses, 52" wide.
71	DTC	LIN21	Linen	Linen-100% Bleached Linen	Linen-100% Bleached Linen	Beautiful soft bleached fabric. Three different weights available. This one is 3.8 oz per square yard with 52x53 Thread Count, 54" wide.
72	DTC	LIN6	Linen	Linen	Linen	100% Linen, Optically Whitened — 6,80z, 54" width.
73	DTC	LIN	Linen	Linen-100% Bleached Linen	Linen-100% Bleached Linen	Beautiful soft bleached fabric. Three different weights available. This one is 4.70z per square yard with 50x54 Thread Count, 54" wide.
74	DTC	KC118	Cotton	Kona Premium Muslin	Kona Premium Muslin 118"	Midweight cotton sheeting. 100% cotton, 4.2 oz per square yard, 118" wide, Thread count is 130 threads per inch x 70 threads per inch.
75	DTC	mac	Cotton	Kona Cotton - PFD	Kona Cotton - PFD 45" wide	A quilter's dream fabric. also good for soft children's clothing, comfy shirts and dresses. The 60x60 thread count gives this even weave fabric a soft luscious feel and great durability. 45" wide. 44 oz/yd.
		KC60	Cotton		Kona Cotton - PFD 60" wide	A quilter's dream fabric. also good for soft children's clothing, comfy shirts and dresses. The 60x60 thread count gives this even weave fabric a soft luscious feel and great durability. 45" wide. 44

						oz/yd.
76	DTC	CVEL	Cotton	Cotton Velveteen - PFD	Cotton Velveteen - PFD	The Velveteen is a woven ICO% cotton with a short close weft pile in imitation Of velvet. Stronger and denser than the rayon Silk velvet and the pile is shorter. 220g/sq meter, 44" wide.
77	DTC	CVO55	Cotton	Cotton Voile	Cotton Voile	Soft, gauzy cotton with a silky wonderful feel. 55" wide 1.9 oz/yd. 80x72 thread count, 30yd bolts.
78	DTC	CST57	Cotton	Cotton Sateen	Cotton Sateen 57"	100% Cotton, mercerized, very soft sheen, 144x66 thread count, 60yd bolts, 57" wide.
79	DTC	CS	Cotton	Cotton Sheeting	Cotton Sheeting	Good quality fabric soft and nice mid-weight. Suitable for all clothing types. 4.20z/sq yd. 55"-58" wide. 60x60 Thread count.
80	DTC	CPOP57	Cotton	Cotton Poplin	Cotton Poplin 57"	100% cotton, mercerized, crisp fabric with a tight weave, 144x68 thread count, 57" wide, bolts are 60 yards.
81	DTC	CPC	Cotton	Cotton Print Cloth Mercerized	Cotton Print Cloth Mercerized	The Best selling cotton fabric. For eyers, dyers, quilters and painters with great quality for finished items (80x80 thread count). Takes dyes and paints particularly well. 45" wide. 3.1 oz/yd
82	DTC	CLF	Cotton	Cotton Lycra PFD	Cotton Lycra PFD	Cotton, Lycra. This is the fabric It's PFD (prepared for dyeing) so it dyes up brilliantly. 60" before shrinkage, 6.3 oz/yd.
83	DTC	CL55	Cotton	Cotton Lawn	Cotton Lawn	100% Cotton, Optically Whitened, 2.0 oz per Square yard. 56/57" wide, thread count 90 threads per inch x 88 threads per inch.
84	DTC	MUS2B	Cotton	Cotton Muslin Bleached	Cotton Muslin 36" - Bleached	Bleached lightweight muslin, with tiny slubs throughout the weave. 100% cotton, oz per square yard, wide. Thread count 68"x68".
85	DTC	7CDB60	Cotton	7 oz'Duck, Bleached	7 oz'Duck, Bleached 60"	Grade "A" Duck. 84 x 29 threads. A little lighter weight. Drapes better than the 10 oz. All dye well. but the natural dyes darker.
		7CDN63	Cotton		7 oz'Duck,Natural 60"	Grade "A" Duck. 84 x 29 threads. A little lighter weight. Drapes better than the 10 oz. All dye well. but the natural dyes darker.

86	DTC	BD-N	Cotton	Bull Denim Natural	Bull Denim Natural	Soft feel and tough as nails. Dyes great. Hats, pants, shorts, bags anywhere you use denim. 60" wide. 10 oz/yd. 50 yard bolts.
		BD-B	Cotton		Bull Denim bleached	Soft feel and tough as nails. Dyes great. Hats, pants, shorts, bags anywhere you use denim. 60" wide. 10 oz/yd. 50 yard bolts.
87	DTC	CCL	Cotton	Combed Cotton Lawn	Combed Cotton Lawn	This is a soft, high quality semi-sheer lawn suitable for blouses. christening gowns, lingerie, and all your fine sewing needs.
88	DTC	CC110	Cotton	Combed Cotton	Combed Cotton 110"	100% Mercerized Soft Cotton, oz per square yard, 110-112" wide. Thread count is 92 threads per inch x 88 threads per inch.
89	DTC	HCG	Cotton	Heavier Cotton Gauze	Heavier Cotton Gauze	Optically whitened, Soft lightly textured. A heavier weight than the cotton bubble gauze. 3.7 oz per square yd, 50" wide, 48 x48 Thread Count.
90	DTC	FT60	Cotton	French Twill	French Twill 60"	Midweight cotton with a tight diagonal weave, 100% cotton, 4.5 oz per square yard. 60" wide, thread count is 132x61
91	DTC	ESS	Cotton	Essex Linen-PFD	Essex Linen- PFD	Linen 145% Cotton Blend. Slightly textured with a linen look. Optically whitened. 5.5 oz per square yd, 52" wide. 51x49 Thread Count.
92	DTC	CVS55	Cotton	Cotton Voile	Cotton Voile	100% Fully Combed Cotton, Optically whitened, 1.4oz per square yard, 57-58" wide, Thread count is 70 x 70 threads per inch.
93	DTC	CJ60	Cotton	Cotton Jersey PFD	Cotton Jersey 60" PFD	100% cotton jersey. PFD ready to dye. 9.5 oz per linear yd. 6 oz./yd. That is a little heavier than the average T-shirt. Used for skirts, dresses, tops and kids' clothing. 60" wide.
94	DTC	CIL	Cotton	Cotton Interlock	Cotton Interlock	Very warm and soft a little stretchy. Perfect for baby wear. Double knit construction makes it thicker & heavier than single knit. 60z/sq. yd, 62" wide.
95	DTC	CG	Cotton	Cotton Bubble Gauze	Cotton Bubble Gauze	Beautiful white all cotton fabric. like a crinkle gauze. Very neat for skirts, tops, anywhere you

						want it light and airy. Heavier gauze is available. 50" wide, 22oz/sq yd.
96	DTC	CF60	Cotton	Combed Cotton Fleece	Combed Cotton Fleece	100% Cotton - 8.70z, 60" width
97	DTC	10CDB60	Cotton	Cotton Duck, Bleached	Cotton Duck, Blch. 10 oz. 45/60"	Grade "A" duck. 76 x 28 threads. Almost has the weight and texture of canvas but the flex and drape of a heavy jacket weight cotton. For strong use. It dyes well, but the natural dyes darker.
		10CDB63	Cotton		Cotton Duck, NATURAL. 10 oz. 63"	Grade "A" duck. 76 x 28 threads. Almost has the weight and texture of canvas but the flex and drape of a heavy jacket weight cotton. For strong use. It dyes well, but the natural dyes darker.
98	Jacquard, Rupert, Gibbon & Spider Inc. (JRGSI)	JAC9701	Cotton	Jackard Inkjet Printable Cotton fabric	Inkjet Printable Cotton fabric	Inkjet fabric sheets 1.5"x11" comes with detachable paper support
99	Silhouette of America Inc.	CTNFAB	Cotton	Sillohuette Inkjet Printable Cotton fabric	Inkjet Printable Cotton fabric	Inkjet fabric sheets 1.5"x11" comes with detachable paper support
100	LumiGram SARL	LUMW01	Polyester	Lumigram optic fiber fabric (white)	Lumigram optic fiber fabric (white)	Lumigram optic fiber fabric (white). Optic fiber diameter is 0.25mm, with a spacing pitch of 1.2mm, horizontally oriented in a matrix of polyester based synthetic fabric (85% PL,15% PA6)
101	Wilton Industries Inc.	500523240	Sucralose /Dextrose	Inkjet Printable Sugar sheets	Inkjet Printable Sugar sheets	Dried inkjet sheets made of food starch-modified (corn), maltodextrin, glycerin, sugar, water, food starch-modified (potato), dextrose, cellulose gum, gum arabic, titanium dioxide (color), polysorbate 60, mono and diglycerides of fatty acids, sorbitan monostearate, potassium sorbate (preservative), artificial flavor, citric acid, sucralose.
102	JRGSI	JAC9702	Silk	Dirty Inkjet	Dirty Inkjet Printable Silk	Inkjet silk fabric sheets 1.5"x11" comes with

				Printable Silk fabric	fabric	detachable paper support
103	JRGSI	JAC9703	Silk	Dirty Semi-transparent Inkjet Printable Silk fabric	Dirty Semi-transparent Inkjet Printable Silk fabric	Thin and semi-transparent inkjet silk fabric sheets 1.5"x11" comes with detachable paper support
104	JRGSI	JAC9701	Cotton	Dirty Inkjet Printable Cotton fabric	Dirty Inkjet Printable Cotton fabric	Inkjet fabric sheets 1.5"x11" comes with detachable paper support
105	Silhouette of America Inc.	CTNFAB	Cotton	Dirty Inkjet Printable Cotton fabric	Dirty Inkjet Printable Cotton fabric	Inkjet fabric sheets 1.5"x11" comes with detachable paper support

Additional descriptions

[00189] FIGs. 6A-6H depict wearable cell-free synthetic biology. FIG. 6A depicts freeze-dried cell-free reactions can be embedded in reaction sachets or chambers that are distributed throughout garments for use by soldiers, clinicians, and first responders. Upon exposure to an external splash, the reactions are rehydrated, activating dormant synthetic gene circuits that detect pathogens, metabolites, and toxins. FIG. 6B depicts a schematic of the layer-by-layer assembly of the wearable devices. Each layer is fabricated from skin-safe silicone elastomer. The FDCF reactions are embedded in a cellulose matrix placed within each chamber. FIG. 6C depicts an array of assembled reaction chambers showing the elasticity (center) and flexibility (right) of the devices. FIG. 6D depicts portals cut into the outermost layer allow sample access, which is rapidly drawn into the reaction chambers through capillary action. The hydrophobic chamber walls prevent inhibitory dilution through lateral diffusion. FIG. 6E-6F depict various types of synthetic biology circuits can be freeze-dried in these wearable devices, including constitutively expressed outputs (6E), transcription factor-regulated circuits for small molecule detection (6F), toehold switches for nucleic acid-sensing (6G), and riboswitches to detect various small molecules (6H). Each graph depicts color deconvoluted values, n=3. Bottom images are representative color images of the wearable device.

[00190] FIGs. 7A-7C depict assembly layers and sample activation of colorimetric wFDCF reactions with constitutive P_{T7}::LacZ module. FIG. 7A depicts the layout of elastomer layers in the colorimetric wFDCF device. FIG. 7B depicts activation of colorimetric prototype reaction chambers using 40 ng/μL constitutive LacZ-T7 plasmid in a 50 μL rehydration splash as compared to FIG. 7C which depicts rehydration with no plasmid. After complete rehydration, PURExpress reactions were conducted at 1.5x concentration. All the reactions were allowed to incubate at 30°C, exposed to the ambient environment, and images were taken every 5 minutes. Color change in one replicate was visible in under 20 min. Each row depicts a representative single-well reaction.

[00191] FIGs. 8A-8C depict sample activation of wFDCF colorimetric devices and bracelet for detection of Ebola virus RNA. FIG. 8A depicts activation of colorimetric Ebola virus DNA toehold wFDCF sensor using a 50 μL splash of dd-H_2O sample containing 300 nM Ebola RNA trigger as compared to control (t = 60 min). FIG. 8B depicts port wicking into reaction chambers containing reaction disks using dd-H_2O fluid splash. Rehydrated paper disks are visibly darker after fluid entry and wicking into the substrate (t = 1 sec after splash). FIG. 8C depicts activation of the wearable colorimetric bracelet with four independent Ebola virus DNA toehold sensors (t

= 25 min). Color change in activated sensor disk is distinguishable within 25-60 min after rehydration, as compared to surrounding controls.

[00192] FIGs. 9A-9G depict design and validation of fluorescent and luminescent freeze-dried cell-free synthetic biology wearables. FIG. 9A depicts details of assembly and activation of fiber-optic based wFDCF module for fluorescence/luminescence output, with a schematic of module layers and components of embedded cell-free reactions. Fiber-optic embedded textiles allow excitation of the samples and detection by sensing emission light. A single layer of blackout cover made of polyester fabric is used to prevent the entry of environmental light into the reaction well. Bottom: An example rehydration event over the device shows the aqueous sample being wicked through the portals and blackout fabric and into internal reaction chambers. FIG. 9B top depicts a diagram showing the layers of the assembled device. Contaminated splashes access the interior of the device through portals in the top layer. FIG. 9B bottom depicts a cross-sectional view of the interior of the device, where two layers of hydrophobically patterned fabric inter-woven with polymeric optic fibers are placed in a coplanar arrangement to allow for rehydration of freeze-dried cell-free reaction components as well as to provide light input/output for excitation and emission signals. Excitation POFs are illuminated with a 447-470 nm LED arrangement, and emission fibers are bundled and aligned with an optical sensor containing an amber filter (for fluorescence readings only) and a collimating lens for magnification. The amber filter can be removed from the device in luminescence mode. FIG. 9C depicts a rapid fluorescent signal after rehydration of wFDCF constitutive sfGFP template as compared to control. Fluorescent signal in-device is statistically distinguishable from the control after 11 min ($P<0.05$). FIG. 9D depicts activation of FDCF riboswitch with 1 mM theophylline in a wearable device as compared to 0 mM theophylline control. Fluorescent signal in-device is statistically distinguishable from the control after 19.5 min ($P<0.05$). FIG. 9E depicts a wearable demonstration of fluorescent aptamer being activated by the presence of 50 μM DFHBI-1T substrate as compared to 0 μM DFHBI-1T control. Fluorescent signal in-device is statistically distinguishable from the control after 24.5 min ($P<0.05$). FIG. 9F depicts luminescence output detected from an HIV toehold sensor with nanoLuciferase operon. HIV RNA trigger was added at 10 μM and was statistically distinguishable from the control after 6 min ($P<0.05$) post-rehydration. FIG. 9G depicts a wearable detection of organophosphate nerve agents using a lyophilized HRP-coupled enzyme sensor rehydrated with 50 mM acetylcholine with and without 3.7 mg/mL paraoxon-ethyl (acetylcholinesterase inhibitor). When the acetylcholinesterase is active, the Amplite-IR substrate is oxidized to generate near-IR fluorescence emission. All images above graphs correspond to time sequences of the recorded POF images in each sensor

demonstration with bundle pictures synchronized with reaction profiles. Each experiment is from three independent reaction chambers each having three fiber optic sensors, for a total of 9 fiber optic outputs. Any fibers that were 1 S.D. below the mean of all nine fiber outputs were excluded from analysis. Scale bars in brightfield images are 250 μm. LED = light-emitting diode, POFs = Polymer Optic Fibers, sfGFP =S uperfolder Green Fluorescent Protein, DFHBI-1T = *difluoro-4-hydroxybenzylidene-1,2-dimethyl-1H-imidazol-5(4H)-one,* HIV = Human Immunodeficiency Virus, AChE = Acetylcholinesterase, ChOx = Choline oxidase, HRP = Horseradish peroxidase, NIR = Near Infrared.

[00193] FIGs. 10A-10D depict concentrating PURE cell-free reactions increases reaction kinetics. FIG. 10A depicts a schematic of reaction concentration through the lyophilization of PURExpress reactions at varying volumes followed by rehydration at a set volume. Using this method, synthetic biology reactions can be concentrated to enhance kinetics through molecular crowding effects or greater density of cell-free components per volume. FIG. 10B depicts representative images of PURE reactions with a LacZ output over one hour, at various concentrations. FIG. 10C depicts quantified PURExpress reactions with a LacZ output in triplicate; the error bars denote standard deviation. FIG. 10D depicts the half-maximal values from curve fitting the data shown in FIG. 10D and indicate that the 1.5x concentrated PURE reaction accelerates the signal output by more than 10 minutes. Error bars are smaller than the data points.

[00194] FIG. 11 depicts Zika DNA Toehold sensor activation in single mercerized cotton thread. Sterile mercerized cotton threads (d = 0.2 mm, L = 1 cm) taken from a DUKAL™ Gauze pad (Dukal Corp., Ronkonkoma, NY) were coiled and deposited into single wells of a flat 384-well black polystyrene plate with a clear glass bottom (Corning Inc., Kennebunk ME). Thread samples were wicked with 2 μL of a solution containing 1x Cell-free PUREXPRESS® in vitro protein synthesis solution (New England Biolabs, Inc., Ipswich, MA), adding 33 nM Zika DNA toehold sensor (sfGFP) for sensing. Samples were frozen using liquid nitrogen and lyophilized for 4 hours. For testing, 2 μL of dd-H_2O with 1.2 μM of freshly made Zika RNA trigger was added to each of the freeze-dried samples, and fluorescence was assessed after 60 minutes under a fluorescence digital microscope Dino-Lite Edge AM4115T-GFBW (Dunwell Tech, Inc., Torrance, CA). These reactions were compared to those occurring in 2 mm disks of WHATMAN™ No. 4 filter paper (GE Healthcare Lifesciences Inc., Chicago, IL). Zika RNA trigger appears to produce a higher fluorescent signal as compared to PURExpress reactions containing no template and reactions with sensor template but with no trigger.

[00195] FIG. 12 depicts antibiotic resistance sensors for *spa*, *ermA* and *mecA* genes using in-wearable sensor demonstrate specific orthogonality. Only reaction chambers with a Cas12a sensor targeting the *S. aureus* virulence factor-encoding *spa*-gene generates a detectable signal within 30 min.

[00196] FIG. 13 depicts POF fabric compatibility with lyophilized transcription-only fluorescent aptamer reactions. The left panel shows a picture of the fabric; the right panel shows a detail magnified view in. POF fabric treated to eliminate RNases was lyophilized with a fluorescent aptamer reaction containing pJL1-F30-2xd-Broccoli aptamer template and an in vitro transcription reaction (HISCRIBE™ T7 Quick High Yield RNA Synthesis Kit; NEB, Ipswich, MA). The lyophilized in-fabric sensors were activated by rehydration with a fluid splash of dd-H_2O spiked with 50 μM of the substrate (5Z)-5-((3,5-Difluoro-4-hydroxyphenyl)methylene)-3,5-dihydro-2-methyl-3-(2,2,2-trifluoroethyl)-4H-imidazol-4-one (DFHBI-1T; Tocris Bioscience, Minneapolis, MN). Upon rehydration, the in vitro transcription reaction generates an RNA aptamer that binds to the DFHBI-1T substrate, generating fluorescence.

[00197] FIG. 14 depicts sensor multiplexing using different fluorescent proteins can be detected in a single device. The top row depicts cell-free reactions demonstrating different fluorescent protein outputs generated after 30 min at 30°C. All tubes were photographed with illumination using an Invitrogen Safe Imager 2.0 G6600 Blue Light Transilluminator (Carlsbad, CA). The bottom row depicts sensor images of fiber topic bundles in (1) brightfield (intense light is placed over the sensor regions to spatially locate each fiber), (2) image when the sensor is dry, (3) image when wFDCF reaction is hydrated but without plasmid (30 min incubation at 30°C), and (4) image when wFDCF reaction is hydrated but with FP plasmids (30 min incubation at 30°C).

[00198] FIGs. 15A-15B depict additional Nanoluciferase (nLuc) luminescence experiments. FIG. 15A depicts dynamic response of a wFDCF Lyme disease RNA toehold switch sensor with luminescence output. In this experiment, 50 μL reactions consisting of 20 μL of NEB cell-free PURExpress® Component A, 15 μL NEB Component B, 2.5 μL NEB murine RNase inhibitor, 19 μL Lyme disease toehold sensor DNA with nLuc reporter (6 ng/μL), 0.5 μL luciferin substrate (Promega Corp., Madison, WI) and 19 μL dd-H_2O. Prepared sensor reactions (50 μL per well) were quickly deposited in-fabric to be snap-frozen and then lyophilized for 4-8 hours within the device. Activation of sensors was achieved by rehydration with a fluid splash of dd-H_2O spiked with 3 μM *B. burgdorferi* trigger RNA freshly made for the positive samples, while 0 μM trigger RNA was used for controls. Luminescence signal from toehold sensor in-device is statistically distinguishable from the control after 13 minutes ($P<0.05$). FIG.15B

depicts dynamic response of a wFDCF HIV RNA toehold switch sensor with luminescence output in comparison to constitutive P_{T7}::nLuc expression as a positive control (+), which was statistically distinguishable from the negative condition after 8 minutes ($P<0.05$). The HIV toehold reaction was prepared in 50 μL batches using 20 μL of NEB cell-free PURExpress® Component A, 15μL NEB Component B, 2.5 μL NEB murine RNase inhibitor, 19 μL HIV toehold sensor DNA template with a nanoLuciferase reporter (6 ng/μL), 0.5 μL luciferin substrate (Promega Corp., Madison, WI) and 19 μL dd-H_2O. Prepared sensor reactions (50 μL per well) were quickly deposited in-fabric to be snap-frozen and then lyophilized for 4-8 hours within the device. Activation of sensors was achieved by rehydration with a fluid splash of dd-H_2O spiked with 10 μM HIV trigger RNA freshly made for the positive samples, while 0 μM HIV trigger RNA was used for controls. The constitutive P_{T7}::nLuc positive control reaction shown was also prepared similarly, but substituting the toehold switch in the plasmid with a T7 promoter. Results were compared to the same reactions run in a 384-well plate and analyzed using a BioTek NEO HTS plate reader (BioTek Instruments, Inc., Winooski, VT) in luminescence mode. Activation of constitutive reaction peaked at ~8 minutes, whereas toehold with 10 μM trigger produced its peak signal at ~15 minutes. Both the wFDCF device tests and the plate reader profiles appeared to be temporally aligned and exhibit analogous signal amplitude differences among reactions.

[00199] FIGs. 16A-16Ddepict fabrication of polymeric optic fiber (POF) fabric for wFDCF. FIG. 16A depicts clean 0.2 mm hydrophilic yarns made of 85% polyester and 15% polyamide were weaved in VELO style along the weft in combination with 0.25 mm un-etched poly(methyl methacrylate) POFs as warp using a standard industrial loom via Dreamlux's process (Samsara S.R.L., Milan, IT). When etched in specific regions, the cladding of POFs can be disrupted to allow for efficient excitation and emission signal collection from fluorescent or luminescent samples rehydrated within the hydrophilic fibers of the fabric. FIG. 16B depicts a three-fiber multi-strip design was achieved with a POF pitch of ~1 mm and intermediate POFs at 5 mm from the strip center for easy cutting. The reaction zone was cut to be ~30 mm in length. The width of the fabric roll was arbitrary, usually above 1 m depending on the used loom. Free POFs can then be detached from the un-weaved side to be bundled together. 16C, A roll of the hydrophilic POF fabric after weaving. FIG. 16D depicts a cut section of the hydrophilic POF fabric with indications in reaction zone and bundle ends.

[00200] FIGs. 17A-17G depicts a fabrication of textile-based wFDCF sensor patch. FIG. 17A depicts a cut strip of hydrophilic POF fabric was laser-etched (5 mm) to disrupt the POF outer cladding in the POFs sections closest to the reaction zone. FIG. 17B depicts examples of

prepared wFDCF fabric-elastomer layers and final assembly into a three-well sensor for garment integration. POFs in these devices were covered with black heat shrink tubing (6 mm). Top elastomer cover features two 5.19 x 1.85 mm curved sample ports instead of three as in the colorimetric prototypes to reduce direct light leakage on top of the POFs that may cause background light detection. FIG. 17C is a schematic of a POF-fabric-elastomer strip for sensing in a single textile layer including two excitation fibers on the sides of an emission fiber. FIG. 17D is a schematic of a double POF-fabric-elastomer strip for sensing with dedicated excitation and emission layers. This design was the one selected for further experiments due to higher hydrophilic fiber content and capacity to immobilize fluid for lyophilization. FIG. 17E is a schematic of a single excitation or emission POF-fabric-elastomer layer overlaid on an applied elastomer pattern for creating the impermeable reaction chambers. FIG. 17F depicts a finalized three-well sensor wFDCF device with heat shrunk POF covers and Luer connectors for interface with a portable spectrometer device. FIG. 17G depicts top and bottom views of a final three-well sensor wFDCF device. The blackout fabric can be seen through the sample wicking ports and serve to prevent environmental light penetration into reaction chambers.

[00201] FIGs. 18A-18B depict textile substrate compatibility testing using synthetic biology reactions and sample colorimetric reaction. FIG. 18A depicts samples of eight fabric types selected as part of the textile screening for wFDCF compatibility. Bottom icons indicate the environmental and hydration conditions that were monitored over time for analysis. FIG. 18B depicts a sample wFDCF colorimetric activation in a 1 x 1 cm cellulose matrix square containing 75 μL of NEB cell-free PUREXPRESS® in vitro protein synthesis solution (New England Biolabs, Inc., Ipswich, MA) with 40 ng/μL constitutive pJL1-LacZ plasmid.

[00202] FIGs. 19A-19B depict textile screening using model constitutive P_{T7}::LacZ assay. FIG. 19A depicts a sample 384-well plate containing triplicates of BSA blocked and unblocked 2 mm discs of 30 different textile types after constitutive P_{T7}::LacZ expression following a 12-hour run for reactions containing 1.8 μL of NEB cell-free PURExpress® in vitro protein synthesis solution (New England Biolabs, Inc., Ipswich, MA) with 40 ng/μL constitutive pJL1-pLacZ plasmid (+) or without plasmid as controls (-). FIG. 19B depicts examples of qualitative traces of colorimetric signals for these different fabric disks using a plate spectrophotometer (420 nm absorbance). While the traces shown here are not normalized across all samples, the increase in signal per cell indicates a color change from yellow to purple. Normalized absorbance values were calculated and used for subsequent analyses.

[00203] FIG. 20 depicts a compilation of normalized functional scoring for colorimetric wF-DCF textile screening. A normalized functionality score was calculated for each of the 103

evaluated fabrics tested for compatibility with freeze-dried PURExpress reaction generating a LacZ output. This score was generated by measuring six key parameters: peak absorbance intensity at 420 nm, reaction rate, time to maximum signal, lag-time, fabric fiber density and in-fabric autofluorescence, and then multiplying normalized scores for each of these measurements, penalizing longer times to maximum signal, long lag-times and high autofluorescence. Dotted line indicates aggregated score value for Whatman No. 4 filter paper. The highest average score was observed in fabric ID#:100 containing 85% Polyester / 15% Polyamide fibers.

[00204] FIGs. 21A-21F depict fabrication of wearable microcontroller system with LED illumination and spectrometric capabilities. FIG. 21A depicts an exploded isometric view of wearable POF spectrometer components with case and electronics. The device electronics are based on a Raspberry Pi Zero W Version 1.3 (Raspberry Pi Foundation, Cambridge, UK), assembled with a PiZ-UpTime battery power board (Alchemy Power Inc., Santa Clara, CA), an environmental sensing module, an LED illumination module, and a flexible camera for imaging. FIG. 21B depicts a photograph of an open assembled device. FIG. 21C depicts a photograph of a fully assembled device ready for imaging. FIG. 21D depicts details of camera used in the device as well as the amber fluorescence emission filter and lens for magnification. Slots at the front of the bottom case fit the camera end, the LED arrangement and a vent for the environmental sensors. FIG. 21E depicts a top view of an assembled device to provide detail of compact electronics arrangement. FIG. 21F depicts an arrangement of wearable POF spectrometer with wireless connectivity in-garment for wFDCF reaction testing.

[00205] FIGs. 22A-22C depict custom mobile application software. FIG. 22A depicts a main window of the developed wFDCD sensor mobile application "Biofabrics" where spectrographic measurements are continuously recorded. Display graphs show independent color channels and bottom icons alert features such as Twitter, email, or messaging as a method of alarm in case of sensor activation. FIG. 22B depicts an environmental window of the mobile application depicts geolocation information as well as recorded measurements of temperature (°C), humidity (%) and CO2 (PPM). FIG. 22C depicts excitation window of the application allows on-the-fly user adjustment of the LED illumination parameters of the four Luxeon Star LEDs installed in the wFDCF device using a Saber Z4 Color Mixing Array (Quadica Developments Inc., Lethbridge, Alberta). LEDs included in the current device were: 447nm, 470nm, 505nm, and 6500K white. This mobile application was developed using blynk.io (Blynk Inc., New York, NY) and the Raspberry Pi communication module. All generated data were recorded in the internal local memory of the wearable device and this application for analysis.

[00206] FIGs. 23A-23J depict validation of CRISPR-based FDCF wearable sensors. FIG. 23A depicts the sensing mechanism of CRISPR-Cas12a system is based on catalytic trans-cleavage of fluorophore-quencher ssDNA probes after activation by an RPA-amplified dsDNA trigger. FIG. 23B depicts wFDCF mecA CRISPR-based sensor exposed to sample containing 100 fM mecA trigger. FIG. 23C depicts wFDCF spa CRISPR-based sensor exposed to 100 fM spa trigger. FIG. 23D depicts wFDCF ermA CRISPR-based sensor exposed to 100 fM ermA trigger. Statistically distinguishable signals ($P<0.05$) were observed after 72, 56 and 78 min for mecA, spa and ermA sensors respectively. FIG. 23E depicts experimental detection of mecA CRISPR-based sensor at 2.7 fM trigger was statistically distinguishable after 75 min ($P<0.05$), corresponding to 10,000 dsDNA-copies per µL. Each experiment is from three independent wells, each having three fiber optic sensors, for a total of 9 fiber optic outputs. Any fibers that were 1 S.D. below the mean of all nine fiber outputs were excluded from analysis. FIG. 23F depicts an orthogonality demonstration of mecA / spa / ermA CRISPR-based multi-sensor wearable. FIG. 23G-23H depict rehydration only yielded activation of sensors when the Cas12a-gRNA sensor was in the presence of its programmed trigger dsDNA. Scale bars are 250 µm. FIG. 23I depict garment-level integration of fabric-based wearable synthetic biology sensors. Distributed continuous sensing of garment activity can be achieved through multi-bundle imaging. FIG. 23J depict Connection of fabric-based module to wearable POF spectrometer with wireless connectivity capabilities. The spectrometer electronics consist of a Raspberry Pi Zero W with a camera module (Raspberry Pi Foundation, Cambridge, UK), as well as LED illumination, environmental sensing, and custom-fabricated shields for battery power. Smartphone application for visualization and alarm of wFDCF sensor activation was based on the blynk.io platform (Blynk Inc., New York, NY) which provides support for Raspberry Pi communication. This application allows for wireless recording of experiments, control of device parameters, as well as environmental and geolocation information.

[00207] FIG. 24 depict limit of detection of wFDCF CRISPR-Cas12a based sensor activated in-fabric. The wFDCF *mecA* CRISPR-based sensor was exposed to various trigger concentrations containing 100, 27, 10, 2.7 and 1 fM *mecA* trigger, to assess in-fabric reaction fluorescence at t = 90 min after fluid entry as compared to controls with a scrambled trigger. Increasing concentrations of trigger lead to an increase in fluorescence signal at the evaluation timepoint as denoted by the recorded mean pixel intensity from POF regions (n=3). A statistically significant difference between the negative control and trigger presence was observed at 90 min only for concentrations equal and above that of 2.7 fM of trigger ($P<0.05$),

which can be considered a limit of detection for this specific trigger, device configuration and evaluation timepoint.

[00208] FIG. 25 depict comparison of Cas13a-based SHERLOCK MRSA RNA-sensing in wFDCF in-fabric prototype against signal in a standardized plate reader. A CRISPR-Cas13a based MRSA SHERLOCK RNA sensor was prepared and freeze-dried over a wearable textile device for testing. This reaction contained Cas13a for ssRNA detection instead of Cas12a for dsDNA detection as reported for the other CRISPR-based sensors. Cell-free reactions were freeze-dried in the wearable devices for 4-8 hours and also freeze-dried in a 384-well plate for comparison in 4 μL reaction aliquots. All reactions contained RNaseAlert substrate, a quenched fluorophore probe that is cleaved by activated Cas13a (Integrated DNA Technologies, Coralville, IA). The wearable sensor was activated with a fluid splash of dd-H_2O containing 20 nM *mecA* RNA trigger, while the plate samples were rehydrated with the same trigger concentrations to the originally deposited reaction volume (4 μL). Reactions were monitored at 30°C for 30 minutes using the wearable optical device or and a BioTek NEO HTS plate reader (BioTek Instruments, Inc., Winooski, VT) in fluorescence mode (Ex. 470 nm / Em. 510 nm). Normalized pixel intensity in the wearable device is comparable in behavior to the results of the kinetic run conducted in the plate reader.

[00209] FIGs. 26A-26D depict integrated wFDCF sample lysis. FIG. 26A depicts detergent combinations for cellular lysis were tested against CRISPR-Cas12a SHERLOCK reactions. Shown are reactions for the SARS-CoV-2 SHERLOCK sensor tested in various detergent dilutions. Based on these results, the 2x dilution was chosen as the optimal lysis buffer. For bacterial samples, the lysis buffer was supplemented with 100 μg/mL of lysozyme for dissolving peptidoglycan and 5% sucrose to create a hyperosmotic environment. FIG. 26B depicts assembly of the wFDCF with lysis: top to bottom; Blackout fabric layer, Disc containing free-dried lysis reagents and lysozyme, dissolvable PVA time delay bridge (edges sealed with elastomer), freeze-dried RPA/SHERLOCK reactions in layer containing POF emission and POF excitation 26C, In-wearable wFDCF mecA sensors containing a lyophilized lysis buffer were challenged with intact *E. coli* cells either containing the target *mecA* gene (+, top images) or a negative control plasmid (-, bottom images). FIG. 26D depicts effectiveness of freeze-dried non-ionic surfactants. The surfactants tested in the top row left to right are Triton X-100, NP-40, and Tween-20. The surfactants tested in the bottom row left to right re Brij-58, Brij-C10, and Brij-S20. All the ionic surfactants show little or no effect on the RFU values. FIG. 26E depicts some ionic surfactants used as freeze-dried lysis reagents. From left to right these are sodium dodecyl sulfate, CHAPS

hydrate, and sodium deoxycholate. Only CHAPs Hydrate shows modest decrease in RFU, Sodium dodecyl sulfate and sodium deoxycholate show immediate impact on RFU.

[00210] FIG. 27A-27D depict bioinspired sample-wicking for textile-based wFDCF synthetic biology devices. FIG. 27A depicts a schematic of the base cover presented for the textile-based wFDCF synthetic biology devices, as well as the underlying biomechanical mechanism of water collection at the areoles of the bunny ears cactus, *Opuntia microdasys*. The high aspect ratio and agglomeration of spikes in these areoles, known as glochids, provide a high wettability gradient, which pins fluid for rapid absorption. FIG. 27B depicts modified cover for the textile-based wFDCF synthetic biology devices with *Opuntia*-inspired wicking ports. The cover features 3D-printed conical spikes (1 mm base diameter) with an aspect ratio of 1:5 arranged concentrically with 1 mm spacing. The cover was fabricated using an elastic photoreactive resin and a stereolithography 3D-printing method using a Form 2 printer (Formlabs Inc., Sommerville, MA), coated with NEVERWET© superhydrophobic coating (NeverWet LLC., Lancaster, PA). Contact angle measurements to confirm hydrophobicity of cover surfaces is also shown. 27C, Five-second time-lapse of the fluid pinning and port wicking exhibited by the device. This demonstration shows that upon fluid splash over the device, fluid rolls through superhydrophobic regions until they encounter the bioinspired ports, which readily pin the fluid, drawing it down into the underlying absorbent fabric layers inside the reaction chamber. FIG. 27D is a photograph of an assembled textile-based wFDCF synthetic biology device including the bioinspired port. Images before and after fluid splash are also shown to evince behavior.

[00211] Table 2. Comparison to other related technology categories

	Wearable Synthetic Biology (This work)	**Current Wearable Devices**	**Point-of-Care Diagnostics**
Wearable Form Factor	YES	YES	NO
Autonomously Functioning	YES	YES	NO
Synthetic Biology Reactions	YES	NO	YES
Molecular Sensing	YES	NO	YES
Pathogen Sensing	YES	NO	YES
Rapid Modular Programmability of Components	YES	NO	YES
Portable Instrumentation	YES	YES	YES

[00212] Table 3. Detailed comparison with other synthetic biology sensor-embedded materials.

	Stretchable Hydrogel-Elastomer Devices with Encapsulated Programmed Cells (Liu, X. et al 2017)	3D Printing of Living Responsive Materials and Devices (Liu, X. et al 2017)	Engineered Bacteria Deposited onto Textiles, Ceramics, and Plastic (Moser, F. et al 2019)	Harnessing the Hygroscopic and Biofluorescent Behaviors of Genetically Tractable Microbial Cells to Design Biohybrid Wearables (Wang, W. et al. 2017)	Programmable CRISPR-Responsive Smart Materials (English, et al. 2020)	wFDCF (This Work)
LOD	100 nM	100 nM	25 mM	n/a	11 aM	17 aM
Sensor Format	Living Cells	Living Cells	Living Cells	Living Cells	Cell-free Freeze-dried Reactions	Cell-free Freeze-dried Reactions
Matrix Material	Hydrogel	Hydrogel	Textiles, Ceramics, Polymer	Polymer	Hydrogel, paper	Textiles, polymer, paper
Shelf-stable	NO	NO	NO	NO	YES	YES
Detection target	Small Molecules	Small Molecules	Small Molecules, Light	Atmospheric Humidity	dsDNA	Hydration, Small Molecules, Proteins, RNA and DNA.
Output	Fluorescent	Fluorescent	Fluorescent	Fluorescent	Fluorescent, Colorimetric, Conductivity	Fluorescent, Colorimetric, Luminescent
Detection time	2 hrs	2-4 hrs	3-18 hrs	minutes	45 min – 24 hrs	6 min – 2 hrs
System Complexity	Simple	Moderate	Moderate	Moderate (Additional Wash Steps)	Moderate	Moderate
Wearable Format	NO	NO	YES	YES	NO	YES

[00213] Table 4. Detailed comparison with point-of-care diagnostic technologies.

Variable	**qPCR**	**Microarrays / Fluorescent barcodes**	**Next Generation Sequencing (NGS)**	**Fluorescence In Situ Hybridization (FISH)**	**Toehold switches**	**SHERLOCK / DETECTR**	**wFDCF (This work)**
Cost (USD)	$0.85	$250.00	$23.00	Expensive	Low-Cost	Low-Cost	Low-Cost (~$100 USD for reusable portable spectrophotometer + ~$1 USD per sensor)
Ease of Use	Trained Specialist	Trained Specialist	Trained Specialist	Trained Specialist	Trained Specialist	Trained Specialist	Autonomous Functioning
Readout	Fluorescence	Fluorescence	Fluorescence	Fluorescence	Visual/ Fluorescence	Visual/ Fluorescence	Visual, Fluorescence, Luminescence and Digital
Sensitivity	aM	aM	aM	aM	fM	aM	aM
Time	~3 Hour	1 Day	>1 Day	4-16 Hour	1-4 Hour	~1-3 Hour	6 min - 2 Hour
Multiplex	YES	YES	YES	YES	YES	YES	YES
Wearable Format	NO	NO	NO	NO	NO	NO	YES

[00214] All patents and other publications identified in the specification and examples are expressly incorporated herein by reference for all purposes. These publications are provided solely for their disclosure prior to the filing date of the present application. Nothing in this regard should be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior invention or for any other reason. All statements as to the date or representation as to the contents of these documents is based on the information available to the applicants and does not constitute any admission as to the correctness of the dates or contents of these documents.

[00215] The terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting of the invention. As used herein, the singular forms "a," "an," and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise. Furthermore, to the extent that the terms "including," "includes," "having," "has," "with," or variants thereof, are used in either the detailed description and/or the claims, such terms are intended to be inclusive in a manner similar to the term "comprising."

[00216] Unless otherwise defined, all terms (including technical and scientific terms) used herein have the same meaning as commonly understood by one of ordinary skill in the art. Furthermore, terms, such as those defined in commonly used dictionaries, should be interpreted as having a meaning that is consistent with their meaning in the context of the relevant art, and will not be interpreted in an idealized or overly formal sense unless expressly so defined herein.

[00217] While various embodiments of the present invention have been described above, it should be understood that they have been presented by way of example only, and not limitation. Numerous changes to the disclosed embodiments can be made in accordance with the disclosure herein, without departing from the spirit or scope of the invention. Thus, the breadth and scope of the present invention should not be limited by any of the above described embodiments. Rather, the scope of the invention should be defined in accordance with the following claims and their equivalents.

[00218] Although the invention has been illustrated and described with respect to one or more implementations, equivalent alterations, and modifications will occur or be known to others skilled in the art upon the reading and understanding of this specification and the annexed drawings. In addition, while a particular feature of the invention may have been disclosed with respect to only one of several implementations, such feature may be combined with one or more other features of the other implementations as may be desired and advantageous for any given or particular application.

[00219] **SEQUENCE LISTING**

Table 5: DNA and RNA sensor sequences used in this study. The sequences presented in this table are SEQ ID NO: 1 to SEQ ID NO: 26 in their order of appearance.

Name	Sequence (5' to 3')	Fig.	Notes
tetO	TCCCTATCAGTGATAGAGATTGACA TCCCTATCAGTGATAGAGATCTAGA AATAATTTTGTTTAACTTTAAGAAG GAGATATACAT	6F	TetR operator region. sfGFP coding region immediately follows.
Zaire Ebola Virus Toehold Switch Sensor	AAGCTGTTGGATATTGTATCAGTCC TTGCTCTGCATGTTATAGTTATGAAC AGAGGAGACATAACATGAACATGC AGAACCATGTTAACCTGGCGGCAGC GCAAAAG*ACCATGATTACGGATTCA*...	6G	Zaire Strain Ebola Toehold Sensor. DNA template is shown. The start of the LacZ coding sequence is shown in italics.
Zaire Ebola Virus RNA trigger (ZD)	AUGCAGAGCAAGGACUGAUACAAU AUCCAACAGCUU	6G	RNA gene fragment trigger of the VP30 gene of Zaire ebolavirus
Theophylline Riboswitch Sensor E (Sensor E)	GGTGATACCAGCATCGTCTTGATGC CCTTGGCAGCACCCTGCTAAGGAGG TAACAACAAGatgagcaaaggtgaagaa*ATG ACCATGATTACGGATTCA*...	6G, 9D	Theophylline riboswitch E sequence. DNA template is shown. Coding sequence begins with a 6-aa sfGFP fragment. The start of the LacZ coding sequence is shown in italics.
F30-2xdBroccoli Aptamer	TTGCCATGTGTATGTGGGAGACGGT CGGGTCCATCTGAGACGGTCGGGTC CAGATATTCGTATCTGTCGAGTAGA GTGTGGGCTCAGATGTCGAGTAGAG TGTGGGCTCCCACATACTCTGATGA TCCAGACGGTCGGGTCCATCTGAGA CGGTCGGGTCCAGATATTCGTATCT GTCGAGTAGAGTGTGGGCTCAGATG TCGAGTAGAGTGTGGGCTGGATCAT TCATGGCAAG	9E	F30-dimeric RNA broccoli aptamer sequence. DNA template is shown. Subcloned from Addgene plasmid #66843 (pET28c-F30-2xdBroccoli).
HIV RNA Toehold switch Sensor (#8)	CCCUUUUCUUUUAAAAUUGUGAAU GAAUACUGCCAUGGACUUUAGAAC AGAGGAGAUAAAGAUGAUGGCAGU AUUAAACCUUGCGGCAGCGCAAAA G	9F	RNA HIV Toehold Sensor
HIV Virus RNA trigger	AUGGCAGUAUUCAUUCACAAUUUU AAAAGAAAAGGG	9F	RNA gene fragment trigger of the HIV retrovirus
Lyme Disease RNA Toehold	GUUGCAGAUGUAUUCCCAUCUUUC CCUGAAUUAUUACGGACUUUAGAA	15A	RNA Lyme Toehold Sensor

switch Sensor	CAGAGGAGAUAAAGAUGGUAAUAA UUCAGAACCUGGCGGCAGCGCAAA AG		
Lyme Disease (*B. burgdorferi*) *ospC* RNA trigger	GGCAAUAUUAAUGACUUUAUUUUU AUUUAUAUCUUGUAAUAAUUCAGG GAAAGAUGGGAAUACAUCUGCAAA UUCUGCUGAUGAGUCUGUUAAAGG GCCUAAUCUUACAGAAAUAAGUAA AAAAAUUACGGAUUCUAA	15A	RNA gene fragment trigger from *B. burgdorferi* bacterium (Lyme's Disease)
CRISPR-Cas12a *mecA* gRNA	GGGUAAUUUCUACUAAGUGUAGAU UUAAAGAAGAUGGUAUGUGG	23B-23E, 24, 12	Cas12a guide RNA targeting single *mecA* gene fragment, was synthesized by in vitro T7 transcription.
CRISPR-Cas12a *mecA* dsDNA trigger	TTTAATTTTGTTAAAGAAGATGGTA TGTGGAAGTTAGATT	23B-23E, 24, 12 23B-23E, 24, 12	Double stranded trigger sequence for Cas12a using *mecA* gRNA.
mecA RPA primer1 (F)	CATTGATCGCAACGTTCAATTTAATT TTGTTAAAG	23B-23E, 24, 12	Forward primer for RPA reaction for Cas12a *mecA* experiments
mecA RPA primer2 (R)	TACGATCAATATGTATGCTTTGGTCT TTCTGCATTCCTG	23B-23E, 24, 12	Reverse primer for RPA reaction for Cas12a *mecA* experiments
CRISPR-Cas12a *ermA* gRNA	GGGUAAUUUCUACUAAGUGUAGAU CUAUUAAUGGUGGAGAUGGA	23B-23E, 12	Cas12a guide RNA targeting single *ermA* gene fragment, was synthesized by in vitro T7 transcription.
CRISPR-Cas12a *ermA*-dsDNA trigger	GCTTTGGGTTTACTATTAATGGTGG AGATGGATATAAAAA	23B-23E, 12	Double stranded trigger sequence for Cas12a using *ermA* gRNA.
ermA RPA primer1 (F)	TCGTTGAGAAGGGATTTGCGAAAAG ATTGC	23B-23E, 12	Forward primer for RPA reaction for Cas12a *ermA experiments*
ermA RPA primer2 (R)	GGATGAAAATATAGTGGTGGTACTT TTTTGAGC	23B-23E, 12	Reverse primer for RPA reaction for Cas12a *ermA experiments*
CRISPR-Cas12a	GGGUAAUUUCUACUAAGUGUAGAU UGGUAAUGCUUGAGCUUUGU	23B-23E,	Cas12a guide RNA targeting single *spa*

spa gRNA		12	gene fragment, was synthesized by in vitro T7 transcription.
CRISPR-Cas12a *spa* dsDNA trigger	TTCACCAGTTTCTGGTAATGCTTGAGCTTTGTTAGCATCT	23B-23E, 12	Double stranded trigger sequence for Cas12a using *spa* gRNA.
spa RPA primer1 *(F)*	AAGAAGCAACCAGCAAACCATGCAGATGC	23B-23E, 12	Forward primer for RPA reaction for Cas12a *spa* experiments
spa RPA primer2 *(R)*	ACCTAACGCTAATGATAATCCACCAAATAC	23B-23E, 12	Reverse primer for RPA reaction for Cas12a *spa* experiments
CRISPR-Cas13a MRSA crRNA	GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACACTCATGCCATACATAAATGGATAGACG	25	Cas13a guide RNA (crRNA) targeting single MRSA gene fragment, was synthesized by in vitro T7 transcription.
CRISPR-Cas13a MRSA Trigger	CGUCUAUCCAUUUAUGUAUGGCAUGAGU	25	Single stranded RNA trigger sequence for Cas13a using MRSA crRNA.
Fluorophore-quencher (FQ) reporter	5'-6FAM/TTATT/IowaBlackFQ	23B-23E, 28C, 24, 12, 26C	Substrate for Cas12a CRISPR-based sensors. When trans-cleaved by cis-activated Cas12a it generates a fluorescent signal.
Zika Toehold switch 27B sensor	TTTCGCTCTATTCTCATCAGTTTCATGTCCTGTGTCGGACTTTAGAACAGAGGAGATAAAGATGGACACAGGACACAACCTGGCGGCAGCGCAAAAGCTGCGTAAACTG*agc*…	11	RNA Zika Toehold Sensor. The start of the sfGFP coding sequence is shown in italics.
Zika Virus RNA trigger	GGGCCAGCACAGUGGGAUGAUCGUUAAUGACACAGGACAUGAAACUGAUGAGAAUAGAGCGAAAGUUGAGAUAACGCCCAAUUCACCAAGAGCCGAAGCCACCCUGGGGGGGUUUGGAAGCCUAGGACUUGAUUGUGAACCGAGGACAGG	11	RNA gene fragment trigger of the Zika single-stranded RNA flavivirus

Notes:

1. All toehold sensors and corresponding trigger RNAs for were in vitro transcribed from DNA templates containing the T7 RNA polymerase promoter: TAATACGACTCACTATA

2. The sequence GGG was added to the 5' end of all toehold sensor RNA and target RNA fragment sequences for efficient expression by T7 RNA polymerase. The Ebola ZD toehold sensor only contains a GG after the T7 promoter. If the RNA sequence began with G or GG, only GG or G, respectively, was added to the 5' end of the sequence.
3. The GGG prefix is not shown in the sensor sequences so that the target RNA binding site can be readily identified, but GGG was always added to the start of each RNA to encourage efficient transcription by the polymerase.
4. The coding sequences of the reporter protein LacZ in the colorimetric sensors were added immediately after the 21-nt linker in the toehold switch RNA sequences starting with the second codon (Threonine) of the wild-type beta-Galactosidase enzyme.
5. Considered Zika virus strains (KU312312, AY632535) have sufficient sequence homology to be detected using the same toehold switch sensors (27B).

Table 6: Reporter sequences used in this study. The sequences presented in this table are SEQ ID NO: 27 to SEQ ID NO: 32 in their order of appearance.

Name (FIG.#)	Sequence (5' to 3')
LacZ Colorimetric Reporter DNA Sequence (6E-6H, 7B,7C, 8A, 10B-10D, 18B, 19A, 19B)	**ATG**ACCATGATTACGGATTCACTGGCCGTCGTTTTACAACGTCGTG ACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACA TCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGAT CGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGCGCTTTG CCTGGTTTCCGGCACCAGAAGCGGTGCCGGAAAGCTGGCTGGAGT GCGATCTTCCTGAGGCCGATACTGTCGTCGTCCCCTCAAACTGGCA GATGCACGGTTACGATGCGCCCATCTACACCAACGTGACCTATCCC ATTACGGTCAATCCGCCGTTTGTTCCCACGGAGAATCCGACGGGTT GTTACTCGCTCACATTTAATGTTGATGAAAGCTGGCTACAGGAAGG CCAGACGCGAATTATTTTTGATGGCGTTAACTCGGCGTTTCATCTGT GGTGCAACGGGCGCTGGGTCGGTTACGGCCAGGACAGTCGTTTGCC GTCTGAATTTGACCTGAGCGCATTTTTACGCGCCGGAGAAAACCGC CTCGCGGTGATGGTGCTGCGCTGGAGTGACGGCAGTTATCTGGAAG ATCAGGATATGTGGCGGATGAGCGGCATTTTCCGTGACGTCTCGTT GCTGCATAAACCGACTACACAAATCAGCGATTTCCATGTTGCCACT CGCTTTAATGATGATTTCAGCCGCGCTGTACTGGAGGCTGAAGTTC AGATGTGCGGCGAGTTGCGTGACTACCTACGGGTAACAGTTTCTTT ATGGCAGGGTGAAACGCAGGTCGCCAGCGGCACCGCGCCTTTCGG CGGTGAAATTATCGATGAGCGTGGTGGTTATGCCGATCGCGTCACA CTACGTCTGAACGTCGAAAACCCGAAACTGTGGAGCGCCGAAATC CCGAATCTCTATCGTGCGGTGGTTGAACTGCACACCGCCGACGGCA CGCTGATTGAAGCAGAAGCCTGCGATGTCGGTTTCCGCGAGGTGCG GATTGAAAATGGTCTGCTGCTGCTGAACGGCAAGCCGTTGCTGATT CGAGGCGTTAACCGTCACGAGCATCATCCTCTGCATGGTCAGGTCA TGGATGAGCAGACGATGGTGCAGGATATCCTGCTGATGAAGCAGA ACAACTTTAACGCCGTGCGCTGTTCGCATTATCCGAACCATCCGCT GTGGTACACGCTGTGCGACCGCTACGGCCTGTATGTGGTGGATGAA GCCAATATTGAAACCCACGGCATGGTGCCAATGAATCGTCTGACCG ATGATCCGCGCTGGCTACCGGCGATGAGCGAACGCGTAACGCGAA TGGTGCAGCGCGATCGTAATCACCCGAGTGTGATCATCTGGTCGCT GGGGAATGAATCAGGCCACGGCGCTAATCACGACGCGCTGTATCG

	CTGGATCAAATCTGTCGATCCTTCCCGCCCGGTGCAGTATGAAGGC GGCGGAGCCGACACCACGGCCACCGATATTATTTGCCCGATGTACG CGCGCGTGGATGAAGACCAGCCCTTCCCGGCTGTGCCGAAATGGTC CATCAAAAAATGGCTTTCGCTACCTGGAGAGACGCGCCCGCTGATC CTTTGCGAATACGCCCACGCGATGGGTAACAGTCTTGGCGGTTTCG CTAAATACTGGCAGGCGTTTCGTCAGTATCCCCGTTTACAGGGCGG CTTCGTCTGGGACTGGGTGGATCAGTCGCTGATTAAATATGATGAA AACGGCAACCCGTGGTCGGCTTACGGCGGTGATTTTGGCGATACGC CGAACGATCGCCAGTTCTGTATGAACGGTCTGGTCTTTGCCGACCG CACGCCGCATCCAGCGCTGACGGAAGCAAAACACCAGCAGCAGTT TTTCCAGTTCCGTTTATCCGGGCAAACCATCGAAGTGACCAGCGAA TACCTGTTCCGTCATAGCGATAACGAGCTCCTGCACTGGATGGTGG CGCTGGATGGTAAGCCGCTGGCAAGCGGTGAAGTGCCTCTGGATGT CGCTCCACAAGGTAAACAGTTGATTGAACTGCCTGAACTACCGCAG CCGGAGAGCGCCGGGCAACTCTGGCTCACAGTACGCGTAGTGCAA CCGAACGCGACCGCATGGTCAGAAGCCGGGCACATCAGCGCCTGG CAGCAGTGGCGTCTGGCGGAAAACCTCAGTGTGACGCTCCCCGCCG CGTCCCACGCCATCCCGCATCTGACCACCAGCGAAATGGATTTTTG CATCGAGCTGGGTAATAAGCGTTGGCAATTTAACCGCCAGTCAGGC TTTCTTTCACAGATGTGGATTGGCGATAAAAAACAACTGCTGACGC CGCTGCGCGATCAGTTCACCCGTGCACCGCTGGATAACGACATTGG CGTAAGTGAAGCGACCCGCATTGACCCTAACGCCTGGGTCGAACGC TGGAAGGCGGCGGGCCATTACCAGGCCGAAGCAGCGTTGTTGCAG TGCACGGCAGATACACTTGCTGATGCGGTGCTGATTACGACCGCTC ACGCGTGGCAGCATCAGGGGAAAACCTTATTTATCAGCCGGAAAA CCTACCGGATTGATGGTAGTGGTCAAATGGCGATTACCGTTGATGT TGAAGTGGCGAGCGATACACCGCATCCGGCGCGGATTGGCCTGAA CTGCCAGCTGGCGCAGGTAGCAGAGCGGGTAAACTGGCTCGGATT AGGGCCGCAAGAAAACTATCCCGACCGCCTTACTGCCGCCTGTTTT GACCGCTGGGATCTGCCATTGTCAGACATGTATACCCCGTACGTCT TCCCGAGCGAAAACGGTCTGCGCTGCGGGACGCGCGAATTGAATT ATGGCCCACACCAGTGGCGCGGCGACTTCCAGTTCAACATCAGCCG CTACAGTCAACAGCAACTGATGGAAACCAGCCATCGCCATCTGCTG CACGCGGAAGAAGGCACATGGCTGAATATCGACGGTTTCCATATG GGGATTGGTGGCGACGACTCCTGGAGCCCGTCAGTATCGGCGGAAT TCCAGCTGAGCGCCGGTCGCTACCATTACCAGTTGGTCTGGTGTCA AAAA**TAA**
GFPmut3B Fluorescent Reporter DNA Sequence (11)	**ATG**CGTAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTG TTGAATTAGATGGTGATGTTAATGGGCACAAATTTTCTGTCAGTGG AGAGGGTGAAGGTGATGCAACATACGGAAAACTTACCCTTAAATTT ATTTGCACTACTGGAAAACTACCTGTTCCGTGGCCAACACTTGTCA CTACTTTCGGTTATGGTGTTCAATGCTTTGCGAGATACCCAGATCAC ATGAAACAGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTACG TACAGGAAAGAACTATATTTTTCAAAGATGACGGGAACTACAAGA CACGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATAGAAT CGAGTTAAAAGGTATTGATTTTAAAGAAGATGGAAACATTCTTGGA CACAAATTGGAATACAACTATAACTCACACAATGTATACATCATGG CAGACAAACAAAAGAATGGAATCAAAGTTAACTTCAAAATTAGAC

	ACAACATTGAAGATGGAAGCGTTCAACTAGCAGACCATTATCAAC AAAATACTCCGATTGGCGATGGCCCTGTCCTTTTACCAGACAACCA TTACCTGTCCACACAATCTGCCCTTTCGAAAGATCCCAACGAAAAG AGAGACCACATGGTCCTTCTTGAGTTTGTAACCGCTGCTGGGATTA CACATGGCATGGATGAACTATACAAAAGGCCTGCAGCAAACGACG AAAACTACGCTTTAGTAGCT**TAA**
sfGFP Fluorescent Reporter DNA Sequence (9C, 9D, 23B-23E)	**ATG**AGCAAAGGTGAAGAACTGTTTACCGGCGTTGTGCCGATTCTGG TGGAACTGGATGGCGATGTGAACGGTCACAAATTCAGCGTGCGTG GTGAAGGTGAAGGCGATGCCACGATTGGCAAACTGACGCTGAAAT TTATCTGCACCACCGGCAAACTGCCGGTGCCGTGGCCGACGCTGGT GACCACCCTGACCTATGGCGTTCAGTGTTTTAGTCGCTATCCGGATC ACATGAAACGTCACGATTTCTTTAAATCTGCAATGCCGGAAGGCTA TGTGCAGGAACGTACGATTAGCTTTAAAGATGATGGCAAATATAAA ACGCGCGCCGTTGTGAAATTTGAAGGCGATACCCTGGTGAACCGCA TTGAACTGAAAGGCACGGATTTTAAAGAAGATGGCAATATCCTGG GCCATAAACTGGAATACAACTTTAATAGCCATAATGTTTATATTAC GGCGGATAAACAGAAAAATGGCATCAAAGCGAATTTTACCGTTCG CCATAACGTTGAAGATGGCAGTGTGCAGCTGGCAGATCATTATCAG CAGAATACCCCGATTGGTGATGGTCCGGTGCTGCTGCCGGATAATC ATTATCTGAGCACGCAGACCGTTCTGTCTAAAGATCCGAACGAAAA AGGCACGCGGGACCACATGGTTCTGCACGAATATGTGAATGCGGC AGGTATTACGTGGAGCCATCCGCAGTTCGAAAAA**TAA**
eforRed Fluorescent Reporter DNA Sequence (14)	**ATG**AGCGTGATTAAACAGGTGATGAAAACCAAACTGCATCTGGAA GGCACCGTTAATGGTCATGATTTCACCATTGAAGGTAAAGGTGAAG GCAAACCGTATGAAGGTCTGCAGCATATGAAAATGACCGTTACCA AAGGTGCACCGCTGCCGTTTAGCGTTCATATTCTGACCCCGAGCCA TATGTATGGTAGCAAACCGTTTAACAAATATCCGGCAGATATCCCG GATTATCACAAACAGAGCTTTCCGGAAGGTATGAGCTGGGAACGT AGCATGATTTTTGAAGATGGTGGTGTTTGTACCGCAAGCAATCATA GCAGCATTAATCTGCAAGAAAACTGCTTCATCTACGACGTGAAATT CCATGGTGTTAATCTGCCTCCGGATGGTCCGGTTATGCAGAAAACC ATTGCAGGTTGGGAACCGAGCGTTGAAACCCTGTATGTTCGTGATG GTATGCTGAAAAGCGATACCGCCATGGTTTTTAAACTGAAAGGTGG TGGTCATCATCGTGTGGATTTCAAAACCACCTACAAAGCAAAAAAA CCGGTTAAACTGCCGGAATTCCATTTTGTTGAACATCGTCTGGAAC TGACCAAACACGATAAAGATTTTACCACCTGGGATCAGCAAGAAG CAGCAGAAGGTCATTTTAGTCCGCTGCCGAAAGCACTGCCG**TAA**
dTomato Fluorescent Reporter DNA Sequence (14)	**ATG**GTGAGCAAGGGCGAGGAGGTCATCAAAGAGTTCATGCGCTTC AAGGTGCGCATGGAGGGCTCCATGAACGGCCACGAGTTCGAGATC GAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCACCCAGACCGCC AAGCTGAAGGTGACCAAGGGCGGCCCCCTGCCCTTCGCCTGGGAC ATCCTGTCCCCCCAGTTCATGTACGGCTCCAAGGCGTACGTGAAGC ACCCCGCCGACATCCCCGATTACAAGAAGCTGTCCTTCCCCGAGGG CTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGTCTGGTG ACCGTGACCCAGGACTCCTCCCTGCAGGACGGCACGCTGATCTACA AGGTGAAGATGCGCGGCACCAACTTCCCCCCCGACGGCCCCGTAAT GCAGAAGAAGACCATGGGCTGGGAGGCCTCCACCGAGCGCCTGTA CCCCCGCGACGGCGTGCTGAAGGGCGAGATCCACCAGGCCCTGAA

	GCTGAAGGACGGCGGCCACTACCTGGTGGAGTTCAAGACCATCTAC ATGGCCAAGAAGCCCGTGCAACTGCCCGGCTACTACTACGTGGACA CCAAGCTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGA ACAGTACGAGCGCTCCGAGGGCCGCCACCACCTGTTCCTGTACGGC ATGGACGAGCTGTACAAG**TAA**
Nanoluciferase Luminescent Reporter DNA Sequence (15A, 15B)	**ATG**GGTCATCACCACCACCATCACGGTGGCGCTAGCGTGTTCACAT TGGAGGACTTTGTAGGGGACTGGCGCCAGACAGCGGGCTACAACC TTGATCAGGTTCTGGAGCAGGGAGGTGTAAGTTCACTTTTCCAGAA TTTGGGTGTGAGTGTCACCCCGATCCAACGTATCGTGCTTTCCGGA GAAAATGGGCTGAAGATCGACATCCATGTTATTATTCCTTATGAAG GGCTTAGCGGAGATCAAATGGGCCAAATCGAAAAGATTTTCAAAG TGGTATATCCTGTTGACGACCATCATTTTAAGGTCATTCTGCATTAC GGAACTTTAGTCATCGACGGCGTCACACCTAACATGATTGACTATT TTGGCCGTCCGTATGAGGGCATCGCAGTGTTTGACGGAAAAAAAAT CACCGTGACAGGGACACTGTGGAACGGCAATAAGATTATCGACGA GCGCCTTATTAACCCAGATGGGTCGCTTTTATTCCGTGTCACTATTA ATGGTGTCACTGGCTGGCGTTTGTGCGAACGCATCCTGGCA**TAA**

Table 7: Sequences for SARS-CoV-2 Molecular Sensing and Isothermal RT-RPA Amplification. The sequences presented in this table are SEQ ID NO: 33 to SEQ ID NO: 86 in their order of appearance.

Toehold Sensor	Performance	Expressed (RNA) Toehold Sequence
SARS2-TH-01	+++	CGAUCCCGCGAAAUUAAUACGACUCACUAUAG GGAUAAUUACCACCAACCUUAGAAUCAAGAUU GUUAGAGGACUUUAGAACAGAGGAGAUAAAG AUGUCUAACAAUCUUACCAUGAUUACGGAUUC ACUGGCCGUC
SARS2-TH-02	+	CGAUCCCGCGAAAUUAAUACGACUCACUAUAG GGGAUUGUUAGAAUUCCAAGCUAUAACGCAGC CUGUAAGGACUUUAGAACAGAGGAGAUAAAG AUGUUACAGGCUGCGACCAUGAUUACGGAUUC ACUGGCCGUC
SARS2-TH-03	+++	CGAUCCCGCGAAAUUAAUACGACUCACUAUAG GGAUAAUUACCACCAACCUUAGAAUCAAGAUU GUUAGAGGACUUUAGAACAGAGGAGAUAAAG AUGUCUAACAAUCUUACCAUGAUUACGGAUUC ACUGGCCGUC
SARS2-TH-04	+++	CGAUCCCGCGAAAUUAAUACGACUCACUAUAG GGACCAUUACAAGGUGUGCUACCGGCCUGAUA GAUUUCGGACUUUAGAACAGAGGAGAUAAAG AUGGAAAUCUAUCAGACCAUGAUUACGGAUUC ACUGGCCGUC
SARS2-TH-05	+	CGAUCCCGCGAAAUUAAUACGACUCACUAUAG GGAACCUUCAACACCAUUACAAGGUGUGCUAC CGGCCUGGACUUUAGAACAGAGGAGAUAAAG AUGAGGCCGGUAGCAACCAUGAUUACGGAUUC ACUGGCCGUC
SARS2-TH-06	+	CGAUCCCGCGAAAUUAAUACGACUCACUAUAG GGCCUUCAACACCAUUACAAGGUGUGCUACCG GCCUGAGGACUUUAGAACAGAGGAGAUAAAG AUGUCAGGCCGGUACACCAUGAUUACGGAUUC

		ACUGGCCGUC
SARS2-TH-07	+	CGAUCCCGCGAAAUUAAUACGACUCACUAUAG GGAAAACCUUCAACACCAUUACAAGGUGUGCU ACCGGCGGACUUUAGAACAGAGGAGAUAAAG AUGGCCGGUAGCACAACCAUGAUUACGGAUUC ACUGGCCGUC
SARS2-TH-09	+	CGAUCCCGCGAAAUUAAUACGACUCACUAUAG GGCUCUGCCAAAUUGUUGGAAAGGCAGAAACU UUUUGUGGACUUUAGAACAGAGGAGAUAAAG AUGACAAAAAGUUUCACCAUGAUUACGGAUUC ACUGGCCGUC
SARS2-TH-10	+	CGAUCCCGCGAAAUUAAUACGACUCACUAUAG GGCAAGAAUCUCAAGUGUCUGUGGAUCACGGA CAGCAUGGACUUUAGAACAGAGGAGAUAAAG AUGAUGCUGUCCGUGACCAUGAUUACGGAUUC ACUGGCCGUC
SARS2-TH-11	-	CGAUCCCGCGAAAUUAAUACGACUCACUAUAG GGCCUGGUUAGAAGUAUUUGUUCCUGGUGUU AUAACACGGACUUUAGAACAGAGGAGAUAAA GAUGGUGUUAUAUCACCAGGACCAUGAUUACG GAUUCACUGGCCGUC
SARS2-TH-12	+	CGAUCCCGCGAAAUUAAUACGACUCACUAUAG GGUAGAACCUGUAGAAUAAACACGCCAAGUAG GAGUAAGGACUUUAGAACAGAGGAGAUAAAG AUGUUACUCCUACUUACCAUGAUUACGGAUUC ACUGGCCGUC
SARS2-TH-13	++	CGAUCCCGCGAAAUUAAUACGACUCACUAUAG GGGCACGUGUUUGAAAAACAUUAGAACCUGU AGAAUAAGGACUUUAGAACAGAGGAGAUAAA GAUGUUAUUCUACAGGACCAUGAUUACGGAU UCACUGGCCGUC
SARS2-TH-14	+	CGAUCCCGCGAAAUUAAUACGACUCACUAUAG GGGUGCCCGCCGAGGAGAAUUAGUCUGAGUCU GAUAACGGACUUUAGAACAGAGGAGAUAAAG AUGGUUAUCAGACUCACCAUGAUUACGGAUUC ACUGGCCGUC
SARS2-TH-15	+	CGAUCCCGCGAAAUUAAUACGACUCACUAUAG GGGCCCGCCGAGGAGAAUUAGUCUGAGUCUGA UAACUAGGACUUUAGAACAGAGGAGAUAAAG AUGAGUUAUCAGACUACCAUGAUUACGGAUUC ACUGGCCGUC
SARS2-TH-16	-	CGAUCCCGCGAAAUUAAUACGACUCACUAUAG GGGCCGAGGAGAAUUAGUCUGAGUCUGAUAA CUAGCGCGGACUUUAGAACAGAGGAGAUAAA GAUGGCGCUAGUUAUCACCAUGAUUACGGAUU CACUGGCCGUC
SARS2-TH-17	-	CGAUCCCGCGAAAUUAAUACGACUCACUAUAG GGGAGGAGAAUUAGUCUGAGUCUGAUAACUA GCGCAUAGGACUUUAGAACAGAGGAGAUAAA GAUGUAUGCGCUAGUUACCAUGAUUACGGAU UCACUGGCCGUC

SARS2-TH-18	-	CGAUCCCGCGAAAUUAAUACGACUCACUAUAG GGGACUAGCUACACUACGUGCCCGCCGAGGAG AAUUAGGGACUUUAGAACAGAGGAGAUAAAG AUGCUAAUUCUCCUCACCAUGAUUACGGAUUC ACUGGCCGUC
SARS2-TH-19	-	CGAUCCCGCGAAAUUAAUACGACUCACUAUAG GGAUUGACUAGCUACACUACGUGCCCGCCGAG GAGAAUGGACUUUAGAACAGAGGAGAUAAAG AUGAUUCUCCUCGGCACCAUGAUUACGGAUUC ACUGGCCGUC
SARS2-TH-20	-	CGAUCCCGCGAAAUUAAUACGACUCACUAUAG GGCAAUGAUGGAUUGACUAGCUACACUACGUG CCCGCCGGACUUUAGAACAGAGGAGAUAAAGA UGGGCGGGCACGUAACCAUGAUUACGGAUUCA CUGGCCGUC
LwaCas13a Sensor		**gRNA Sequence**
SARS2-Lwa13-01	++	GAUUUAGACUACCCCAAAAACGAAGGGGACUA AAACCACCAACCUUAGAAUCAAGAUUGUUAGA
SARS2-Lwa13-02	+++	GAUUUAGACUACCCCAAAAACGAAGGGGACUA AAACCCAUUACAAGGUGUGCUACCGGCCUGAU
SARS2-Lwa13-03	+++	GAUUUAGACUACCCCAAAAACGAAGGGGACUA AAACAACCUUCAACACCAUUACAAGGUGUGCU
SARS2-Lwa13-04	+++	GAUUUAGACUACCCCAAAAACGAAGGGGACUA AAACACACCAUUACAAGGUGUGCUACCGGCCU
SARS2-Lwa13-05	+++	GAUUUAGACUACCCCAAAAACGAAGGGGACUA AAACAAAACCUUCAACACCAUUACAAGGUGUG
SARS2-Lwa13-06	+++	GAUUUAGACUACCCCAAAAACGAAGGGGACUA AAACGUAACAAUUAAAACCUUCAACACCAUUA
SARS2-Lwa13-07	-	GAUUUAGACUACCCCAAAAACGAAGGGGACUA AAACAAUUGUUGGAAAGGCAGAAACUUUUUG U
SARS2-Lwa13-08	+++	GAUUUAGACUACCCCAAAAACGAAGGGGACUA AAACCAAGAAUCUCAAGUGUCUGUGGAUCACG
SARS2-Lwa13-09	+++	GAUUUAGACUACCCCAAAAACGAAGGGGACUA AAACGAAGUAUUUGUUCCUGGUGUUAUAACA C
SARS2-Lwa13-10	+++	GAUUUAGACUACCCCAAAAACGAAGGGGACUA AAACGUAGAAUAAACACGCCAAGUAGGAGUA A
SARS2-Lwa13-11	++	GAUUUAGACUACCCCAAAAACGAAGGGGACUA AAACGCACGUGUUUGAAAAACAUUAGAACCUG
SARS2-Lwa13-12	++	GAUUUAGACUACCCCAAAAACGAAGGGGACUA AAACGUGCCCGCCGAGGAGAAUUAGUCUGAGU
SARS2-Lwa13-13	++	GAUUUAGACUACCCCAAAAACGAAGGGGACUA AAACGCCCGCCGAGGAGAAUUAGUCUGAGUCU
SARS2-Lwa13-14	-	GAUUUAGACUACCCCAAAAACGAAGGGGACUA AAACGCCGAGGAGAAUUAGUCUGAGUCUGAU A
SARS2-Lwa13-15	-	GAUUUAGACUACCCCAAAAACGAAGGGGACUA AAACGAGGAGAAUUAGUCUGAGUCUGAUAAC

		U
SARS2-Lwa13-16	-	GAUUUAGACUACCCCAAAAACGAAGGGGACUA AAACGCUACACUACGUGCCCGCCGAGGAGAAU
SARS2-Lwa13-17	-	GAUUUAGACUACCCCAAAAACGAAGGGGACUA AAACACACUACGUGCCCGCCGAGGAGAAUUAG
SARS2-Lwa13-18	-	GAUUUAGACUACCCCAAAAACGAAGGGGACUA AAACCUACGUGCCCGCCGAGGAGAAUUAGUCU
LbaCas12a Sensor		**gRNA Sequence**
SARS2-Lba12a-01	+++	CGAUCCCGCGAAAUUAAUACGACUCACUAUAG GGUAAUUUCUACUAAGUGUAGAUAGUGUUAU GGAGUGUCUCCUACUA
SARS2-Lba12a-02	+++	CGAUCCCGCGAAAUUAAUACGACUCACUAUAG GGUAAUUUCUACUAAGUGUAGAUCAGGCUGC GUUAUAGCUUGGAAUU
SARS2-Lba12a-03	+++	CGAUCCCGCGAAAUUAAUACGACUCACUAUAG GGUAAUUUCUACUAAGUGUAGAUGCAGAGAC AUUGCUGACACUACUG
SARS2-Lba12a-04	+++	CGAUCCCGCGAAAUUAAUACGACUCACUAUAG GGUAAUUUCUACUAAGUGUAGAUAUAGGGGC UGAACAUGUCAACAAC
SARS2-Lba12a-05	+++	CGAUCCCGCGAAAUUAAUACGACUCACUAUAG GGUAAUUUCUACUAAGUGUAGAUUAAUUAGA GGUGAUGAAGUCAGAC
SARS2-Lba12a-06	+++	CGAUCCCGCGAAAUUAAUACGACUCACUAUAG GGUAAUUUCUACUAAGUGUAGAUCAGGCUGC GUUAUAGCUUGGAAUU
SARS2-Lba12a-07	+++	CGAUCCCGCGAAAUUAAUACGACUCACUAUAG GGUAAUUUCUACUAAGUGUAGAUAACUGAAA UCUAUCAGGCCGGUAG
SARS2-Lba12a-08	+++	CGAUCCCGCGAAAUUAAUACGACUCACUAUAG GGUAAUUUCUACUAAGUGUAGAUGCAGAGAC AUUGCUGACACUACUG
SARS2-Lba12a-09	+++	CGAUCCCGCGAAAUUAAUACGACUCACUAUAG GGUAAUUUCUACUAAGUGUAGAUGUAGGAGA CACUCCAUAACACUUA
SARS2-Lba12a-10	+++	CGAUCCCGCGAAAUUAAUACGACUCACUAUAG GGUAAUUUCUACUAAGUGUAGAUUCUGACUU CAUCACCUCUAAUUAC
SARS2-Lba12a-11	+++	CGAUCCCGCGAAAUUAAUACGACUCACUAUAG GGUAAUUUCUACUAAGUGUAGAUAAAAACAU UAGAACCUGUAGAAUA
RT-RPA Primers		**Oligo Sequence**
RPA-06-F4	+++	GCAAACTGGAAAGATTGCTGATTATAATTATAA ATTACC
RPA-06-R4	+++	CCTGATAGATTTCAGTTGAAATATCTC
RPA-06-R3	+++	CCTTCAACACCATTACAAGGTGTGCTACC
SARS-CoV-2 S-gene amplification primers including any part or fragment of the following nucleic sequences or their respective reverse complementing sequence:		

tcaaacttctaactttagagtccaaccaacagaatctattgttagatttcctaatattacaaacttgtgccctttggtgaagtttttaacgccacca gatttgcatctgtttatgcttggaacaggaagagaatcagcaactgtgttgctgattattctgtcctatataattccgcatcattttccacttttaagt gttatggagtgtctcctactaaattaaatgatctctgctttactaatgtctatgcagattcatttgtaattagaggtgatgaagtcagacaaatcgct ccagggcaaactggaaagattgctgattataattataaattaccagatgattttacaggctgcgttatagcttggaattctaacaatcttgattcta aggttggtggtaattataattacctgtatagattgtttaggaagtctaatctcaaacctttgagagagatatttcaactgaaatctatcaggccgg tagcacaccttgtaatggtgttgaaggttttaattgttactttcctttacaatcatatggtttccaacccacttatggtgttggttaccaaccatacag agtagtagtactttcttttgaacttctacatgcaccagcaactgtttgtggacctaaaaagtctactaatttggttaaaaacaaatgtgtcaatttca acttcaatggtttaacaggcacaggtgttcttactgagtctaacaaaaagtttctgcctttccaacaatttggcagagacattgctgacactactg atgctgtccgtgatccacagacacttgagattcttgacattacaccatgttcttttggtggtgtcagtgttataacaccaggaacaaatacttctaa ccaggttgctgttctttatcagggtgttaactgcacagaagtccctgttgctattcatgcagatcaacttactcctacttggcgtgtttattctacag gttctaatgtttttcaaacacgtgcaggctgtttaataggggctgaacatgtcaacaactcatatgagtgtgacatacccattggtgcaggtatat gcgctagttatcaga
* for the RNA sequences, the respective dsDNA, template ssDNA and reverse complement ssDNA sequences can be used.

CLAIMS

WHAT IS CLAIMED IS:

1. An aqueous solution-activated sensor fabric comprising:

an excitation plastic optical fiber (POF) and an emission POF combined with a porous hydrophilic material into a flat web structure, and

an freeze-dried cell-free (FDCF) synthetic biological component in at least a portion of the web structure.

2. The aqueous solution-activated sensor fabric according to claim 1, wherein the web structure is a woven structure including a first layer wherein the excitation POF is weaved in a warp direction, and the porous hydrophilic material is woven in the weft direction.

3. The aqueous solution-activated sensor fabric according to claim 2, wherein the excitation POF includes a plurality of substantially parallel POFs.

4. The aqueous solution-activated sensor fabric according to claim 2 or 3, wherein the web structure is a woven structure including a second layer wherein the emission POF is weaved in a warp direction, and the porous hydrophilic material is woven in the weft direction.

5. The aqueous solution-activated sensor fabric according to any one of claims 1 to 4, wherein the emission POF includes a plurality of substantially parallel POFs.

6. The aqueous solution-activated sensor fabric according to any one of claims 1 to 5, wherein the excitation POF and the emission POF are etched to remove a portion of outer cladding.

7. The aqueous solution-activated sensor fabric according to any one of claims 1 to 6, wherein the FDCF synthetic biological component is spatially contained by being surrounded by patterns of a hydrophobic material, the hydrophobic material including a port exposing a portion of the synthetic biological component to the environment.

8. The aqueous solution-activated sensor fabric according to any one of claims 1 to 7, wherein the porous hydrophilic material comprises one or more of a cellulose, starch, maltodextrin, glycerine, sugar, sucralose, dextrose, gum arabic, cotton, wool, silk, rayon, hemp, spandex/lycra/elastane, polyester, polyamide, linen, nylon, or a blend thereof.

9. The aqueous solution-activated sensor fabric according to any one of claims 1 to 8, wherein the porous hydrophilic material includes a first material that is combined with the excitation POF and a second material combined the emission POF.

10. The aqueous solution-activated sensor fabric according to any one of claims 1 to 9, wherein a first end of the excitation POF, and a first and of the emission POF are treated with a reflective coating.

11. An aqueous solution-activated sensor comprising:

a chamber formed in a flexible material;

a port fluidly connecting an exterior surface of the flexible material to an interior of the chamber, the port allowing an aqueous solution in contact with the exterior surface to be wicked to the interior;

a first UV-Vis light-transmitting medium providing a first optical connection from the interior of the chamber to an exterior of the chamber; and

freeze-dried cell-free (FDCF) synthetic biological components disposed in the chamber,

the FDCF synthetic biological components being hydrated upon exposure to the aqueous solution to form rehydrated synthetic biological components formulated to provide an optical signal transmittable through the light transmitting medium responsive to the presence or absence of a triggering compound in the aqueous solution wicked to the interior of the chamber.

12. The aqueous solution-activated sensor according to claim 11, wherein the chamber volume is between about 0.1 μL and about 500 μL

13 The aqueous solution-activated sensor according to claim 12, wherein the chamber volume is between about 1 μL and about 150 μL-spec.

14. The aqueous solution-activated sensor according to any one of claims 11 to 13, wherein the port includes an opening on the exterior surface that is between 0.1μm^2 and 50 mm^2.

15. The aqueous solution-activated sensor according to any one of the claims 11 to 14, wherein the synthetic biological components include toehold sensor components, transcription-factor sensor components, aptameric sensor components, enzyme sensor components, antibody sensor components, CRISPR DNA sensor components, CRISPR RNA sensor components, ribonucleoprotein sensor components, and combinations thereof.

16. The aqueous solution-activated sensor according to any one of claims 11 to 15, wherein the rehydrated synthetic biological components have a concentration between 1 and 2.4 times a specified concentration.

17. The aqueous solution-activated sensor according to any one of the claims 11 to 16, wherein the synthetic biological components provide the optical signal when activated with the triggering compound by synthesizing, activating, or suppressing, a colored, fluorescent or luminescent protein.

18. The aqueous solution-activated sensor according to any one of the claims 11 to 17, wherein the triggering compound is an RNA, a ss-DNA, a DNA, an oligonucleotide, a protein, a peptide, an aptamer, an antibody, an antigen, a small molecule or combinations thereof.

19. The aqueous solution-activated sensor according to any one of the claims 11 to 18, further comprising a dried lysate disposed in the chamber.

20. The aqueous solution-activated sensor according to claim 19, wherein the lysate includes one or more of Triton X-100, NP-40, Tween-20, any of Brij non-ionic surfactants, CHAPS hydrate, lysozyme, and disaccharides or polysaccharides such as sucrose, mannitose, or trehalose.

21. The aqueous solution-activated sensor according to claims 19 or 20, wherein the dried lysate is disposed in the chamber between the port and the FDCF biological components.

22. The aqueous solution-activated sensor according to any one of claims 19 to 21, wherein the dried lysate is adsorbed on a porous hydrophilic material.

23. The aqueous solution-activated sensor according to any one of claims 19 to 22, further comprising a dissolvable membrane disposed between the dried lysate and the FDCF biological components.

24. The aqueous solution-activated sensor according to claim 23, wherein the dissolvable membrane incudes polyvinyl alcohol (PVA), sugars such as sucrose, inorganic salts, patterned hydrophobic solids, or other compounds to provide a fluidic delay based on solubility.

25. The aqueous solution-activated sensor according to any one of claims 19 to 24, further comprising a barrier disposed between the dried lysate and the FDCF biological compounds, the barrier including a tortuous channel fluidly connecting the dried lysate and the FDCF biological compounds.

26. The aqueous solution-activated sensor according to any one of the claims 11 to 25, further comprising a porous hydrophilic material in the chamber.

27. The aqueous solution-activated sensor according to claim 26, wherein the porous hydrophilic material comprises one or more of cellulose, starch, maltodextrin, glycerine, sugar, sucralose, dextrose, gum arabic, cotton, wool, silk, rayon, hemp, spandex/lycra/elastane, polyester, polyamide, linen, nylon, or other synthetic or natural fibers, or combinations thereof.

28. The aqueous solution-activated sensor according to claim 26 or 27, wherein the porous hydrophilic material is treated with a blocking agent that either covalently bonds with the material or uses non-covalent interactions to block the material with a desired physiochemical characteristic.

29. The aqueous solution-activated sensor according to any one of claims 11 to 28, wherein at least a portion of the flexible material is opaque to UV-Vis light.

30. The aqueous solution-activated sensor according to any one of claims 11 to 29, wherein the flexible material comprises a bottom layer defining a bottom wall of the chamber, a middle layer defining a side wall of the chamber, and a top layer defining a top wall of the chamber.

31. The aqueous solution-activated sensor according to claim 30, wherein one or more of the bottom, middle, or top layer comprises an elastomeric material.

32. The aqueous solution-activated sensor according to claim 31, wherein the elastomeric material is one or more of ethylene propylene diene monomer (EPDM) rubber, a silicone, a neoprene rubber, a natural rubber, a nitrile rubber, a butyl rubber, a thermoplastic elastomer, or any hydrophobic elastomer.

33. The aqueous solution-activated sensor according to claim any one of claims 30 to 32, wherein the UV-Vis light transmitting medium comprises at least a portion of the top wall.

34. The aqueous solution-activated sensor according to any one of claims 30 to 33, wherein the top layer is optically transparent to UV-Vis light.

35. The aqueous solution-activated sensor according to any one of claims 11 to 34, further comprising a second UV-Vis light transmitting medium providing a second optical connection from the interior of the chamber to the exterior of the chamber.

36. The aqueous solution-activated sensor according to claim 35, wherein the first UV-Vis light transmitting medium is an emission plastic optical fiber (POF), and the second UV-Vis light transmitting medium is an excitation POF.

37. The aqueous solution-activated sensor according to claim 36, wherein a portion of an outer cladding of the emission POF is removed to provide the first optical connection from the interior of the chamber, and a portion of an outer cladding of the excitation POF is removed to provide the second optical connection to the interior of the chamber.

38. The aqueous solution-activated sensor according to claim 36 or 37, wherein the emission POF and excitation POF are connected to a spectrophotometer.

39. The aqueous solution-activated sensor according to any one of claims 36 to 38, wherein the emission POF and the excitation POF are combined with a porous hydrophilic material.

40. The aqueous solution-activated sensor according to claim 39, wherein the emission POF and the excitation POF are interwoven with the porous hydrophilic material providing a woven fabric.

41. The aqueous solution-activated sensor according to claim 40, wherein the emission POF is interwoven with a first portion of porous hydrophilic material providing a first woven fabric,

and the excitation POF is interwoven with a second portion of the porous hydrophilic material providing a second woven fabric.

42. The aqueous solution-activated sensor according to any one of claims 39 to 41, wherein the porous hydrophilic material comprises one or more of a cellulose, starch, maltodextrin, glycerine, sugar, sucralose, dextrose, gum Arabic, cotton, wool, silk, rayon, hemp, spandex/lycra/elastane, polyester, polyamide, linen, nylon, or a blend thereof.

43. The aqueous solution-activated sensor according to any one of claims 39 to 42, wherein at least a portion of the porous hydrophilic material is embedded in the flexible material.

44. The aqueous solution-activated sensor according to any one of claims 39 to 43, wherein the porous hydrophilic material is treated with a blocking agent.

45. The aqueous solution-activated sensor according to any one of claims 11 to 43, wherein the sensor is configured as a portion of a wearable item.

46. The aqueous solution-activated sensor according to claim 45, wherein the wearable item is a shirt; a jacket; pants; a skirt; a laboratory coat; a full-body garment; an exterior worn armor; a wrist, arm, head or ankle band; a scarf; gloves; socks; shoes or boots; a necklace; a ring; a hat; a helmet; a brooch; a face mask; a patch; or other wearable garments.

47. The aqueous solution-activated sensor according to any one of claims 11 to 46, further comprising a plurality of conical spikes perpendicular to the exterior surface and proximate to the port.

48. A method for making an aqueous solution-activated sensor, the method comprising:

providing a layer of a first material, a portion of a top surface of the first material defining a bottom wall of a chamber;

providing a layer of a second material on the top surface of the first material, the second material including a first continuous open space on the portion of the top surface defining the bottom wall of the chamber, the second material defining a side wall of the chamber;

providing a layer of a third material on a top surface of the second material, the third material including a second continuous open space disposed above the chamber, the third material defining a top wall of the chamber including a port defined by the second continuous open space;

adding synthetic biological components into the chamber; and

freeze drying the synthetic biological components.

49. The method according to claim 48, wherein the synthetic biological components include toehold sensor components, transcription-factor sensor components, aptameric sensor

components, enzyme-based sensor components, antibody sensor components, CRISPR DNA sensor components, CRISPR RNA sensor components, and any ribonucleoprotein sensor components.

50. The method according to claim 48 or 49, further including adding a prokaryotic or eukaryotic cell lysate into the chamber.

51. The method according to claim 50, wherein the lysate is either dried or freeze-dried.

52. The method according to claim 50 or 51, wherein the lysate is positioned in the chamber between the port and the synthetic biological components.

53. The method according to any one of claims 50 to 52, wherein the lysate is absorbed on a hydrophilic material.

54. The method according to any one of claims 50 to 53, further comprising placing a dissolvable membrane or material between the lysate and the synthetic biological components.

55. The method according to claim 54, wherein the dissolvable membrane includes PVA.

56. The method according to any one of claims 48 to 55, wherein providing the layer of the first material, the second material and the third material occur in any order to define the chamber.

57. The method according to any one of claims 48 to 56, further comprising curing any one or more of the first material, the second material, and the third material prior to, during, or after providing the first material, second material, or third material as a layer.

58. The method according to any one of claims 48 to 56, further comprising solidifying any one or more of the first material, the second material, and the third material from a molten state prior to, during, or after providing the first material, second material, or third material as a layer.

59. The method according to any one of claims 48 to 58, further comprising forming, by a polymerization reaction, any one or more of the first material, the second material, and the third material from monomeric precursors, during, or after providing the first material, second material, or third material as a layer.

60. The method according to any one of claims 48 to 59, wherein the first material, the second material, and the third material form a flexible planar sensor.

61. The method according to any one of claims 48 to 60, wherein the first material, the second material, and the third material comprise an elastomeric material.

62. The method according to any one of claims 48 to 61, wherein the third material is transparent to UV-Vis light for at least in a portion of the third material disposed above the chamber, thereby providing a UV-Vis light transmitting medium.

63. The method according to any one of claims 48 to 62, further comprising adding a porous hydrophilic material to the chamber.

64. The method according to any one of claims 48 to 63, further comprising including an excitation plastic optical fiber (POF) and an emission POF with the second material, the excitation POF and the emission POF extending through the chamber wall, and out of the second material.

65. The method according to claim 64, wherein the excitation POF and the emission POF are combined with a porous hydrophilic material.

66. The method according to claim 65, wherein the excitation POF and the emission POF are interwoven with the porous hydrophilic material providing a woven fabric.

67. The method according to claim 65 or 66, wherein the second material comprises a first layer including the second material and the excitation POF, and a second layer including the second material and the emission POF.

68. The method according to claim 67, wherein the first layer is placed on the second layer to provide the second layer.

69. The method according to any one of claims 64 to 68, wherein;

a first end of the excitation POF is treated with a reflective coating, and a second end of the excitation POF is connected to an excitation source,

a first end of the emission POF is treated with a reflective coating, and a second end of the emission POF is connected to an emission detector.

70. The method according to any one of claims 64 to 69, wherein a portion of the excitation POF and a portion of the emission POF are chemically, physically, or optically etched to remove an outer cladding, said portions positioned in the chamber.

71. The method according to claim 70, wherein the outer cladding is etched using a laser.

72. The method according to any one of claims 64 to 71, further comprising including an opaque barrier between the port and both of the emission POF and excitation POF, thereby blocking light transmission from the port to the emission POF and excitation POF.

73. The method according to any one of claims 48 to 72, further comprising forming a plurality of cones proximate to the port and extending perpendicularly from a top external surface of the third material.

74. The method according to claim 73, wherein the cones are 3D printed.

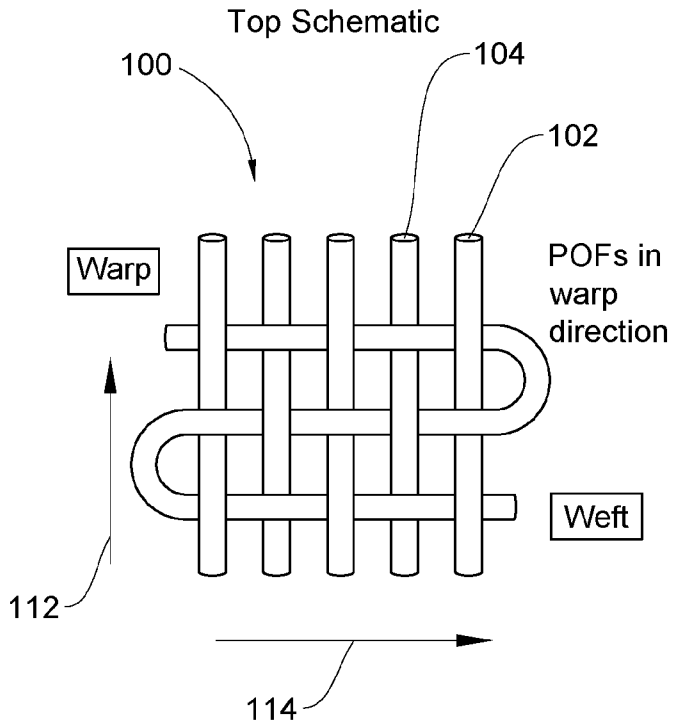


FIG. 1A

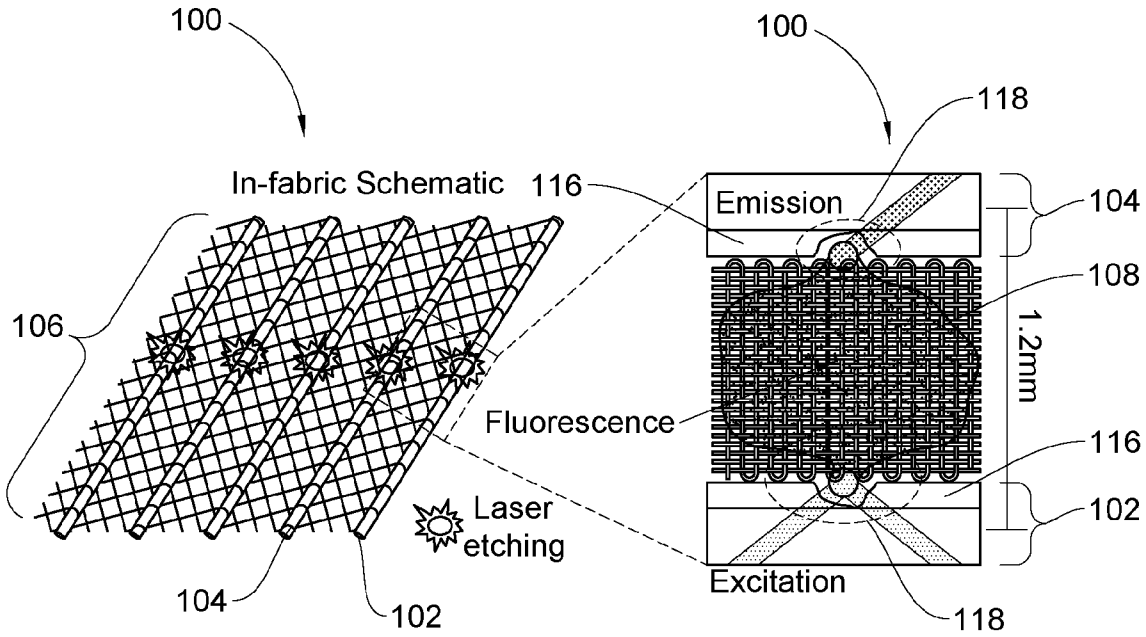


FIG. 1B

FIG. 1C

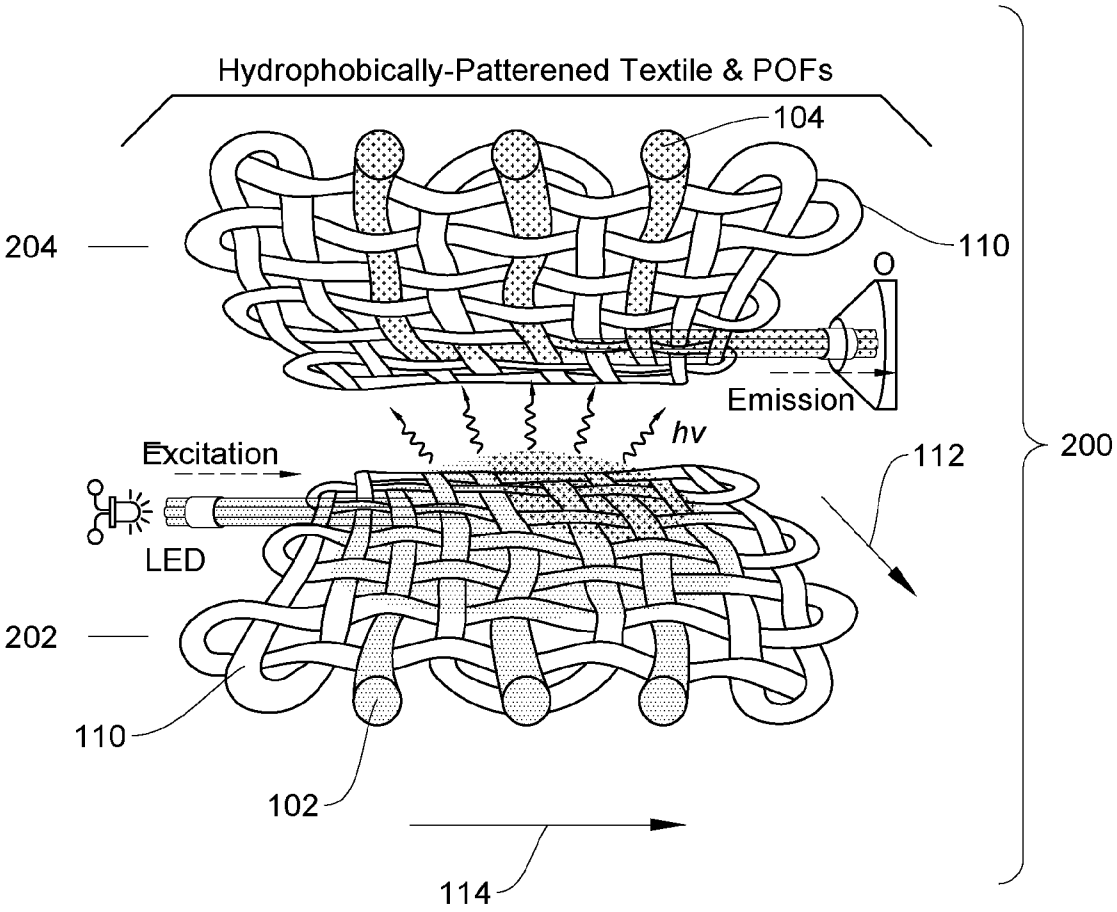

Hydrophobically-Patterened Textile & POFs
104
204
110
O
Emission
hv
Excitation
112
200
LED
202
110
102
114

FIG. 2

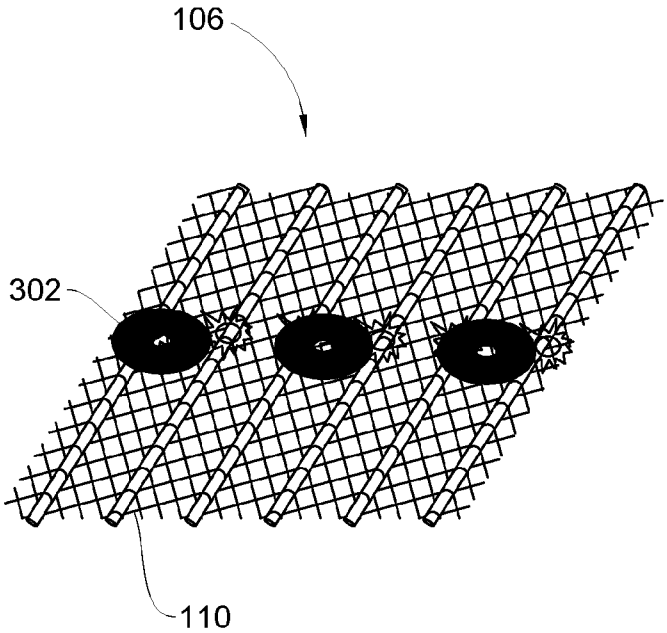


FIG. 3A

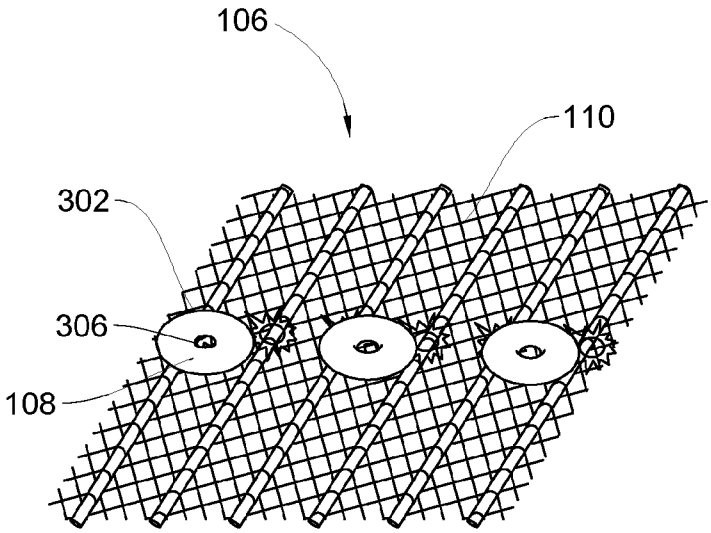


FIG. 3B

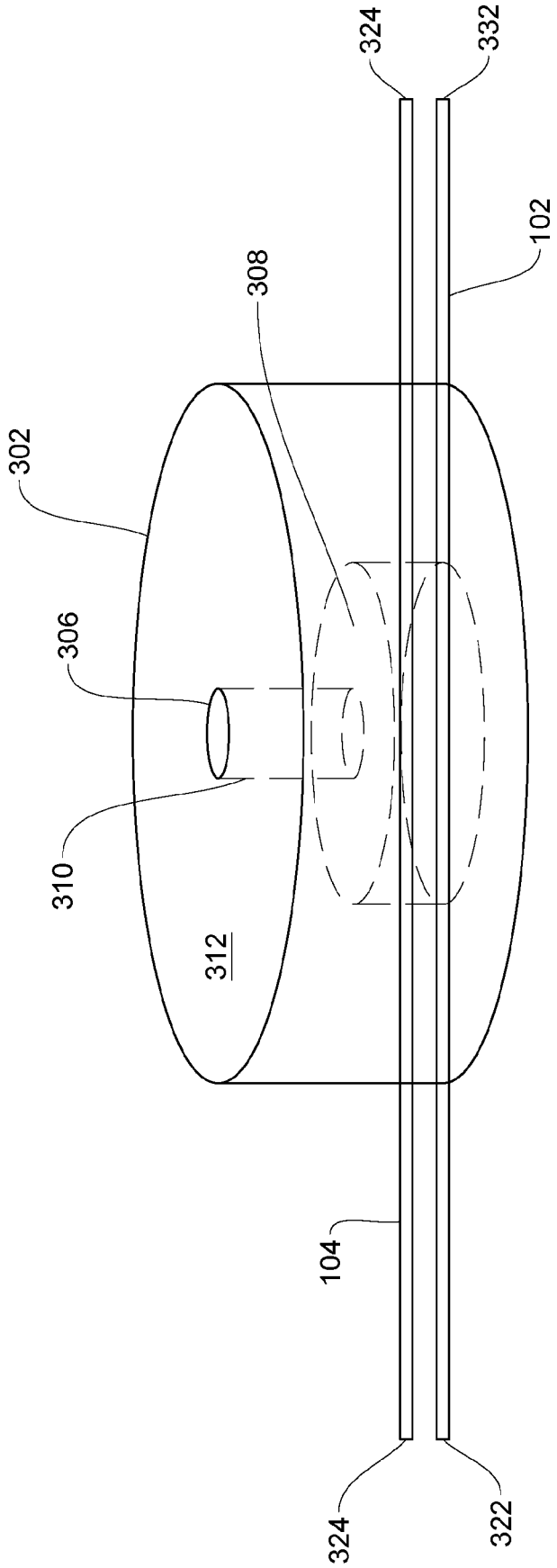


FIG. 3C

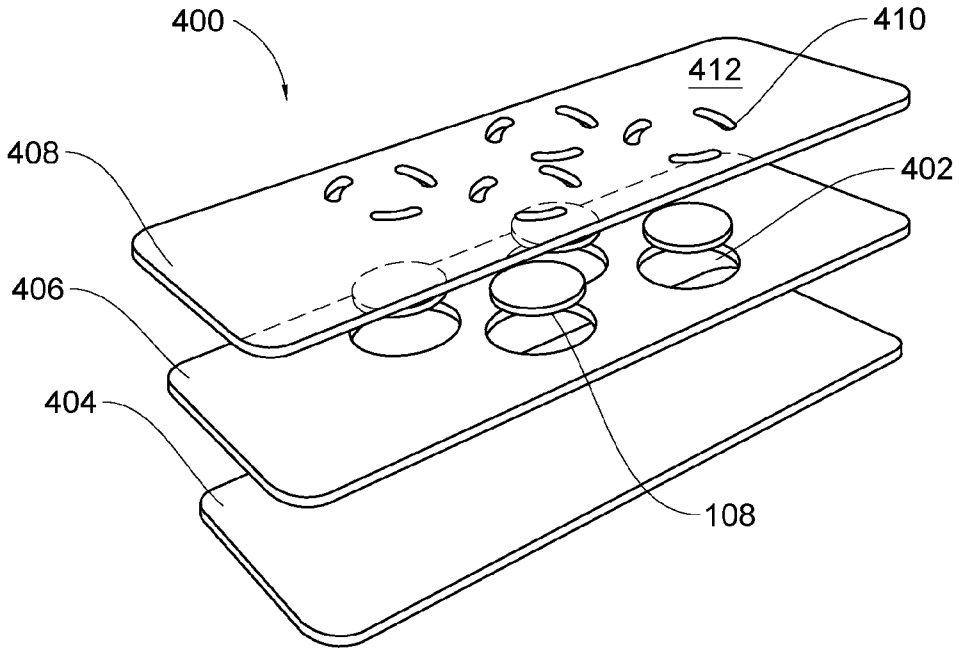


FIG. 4A

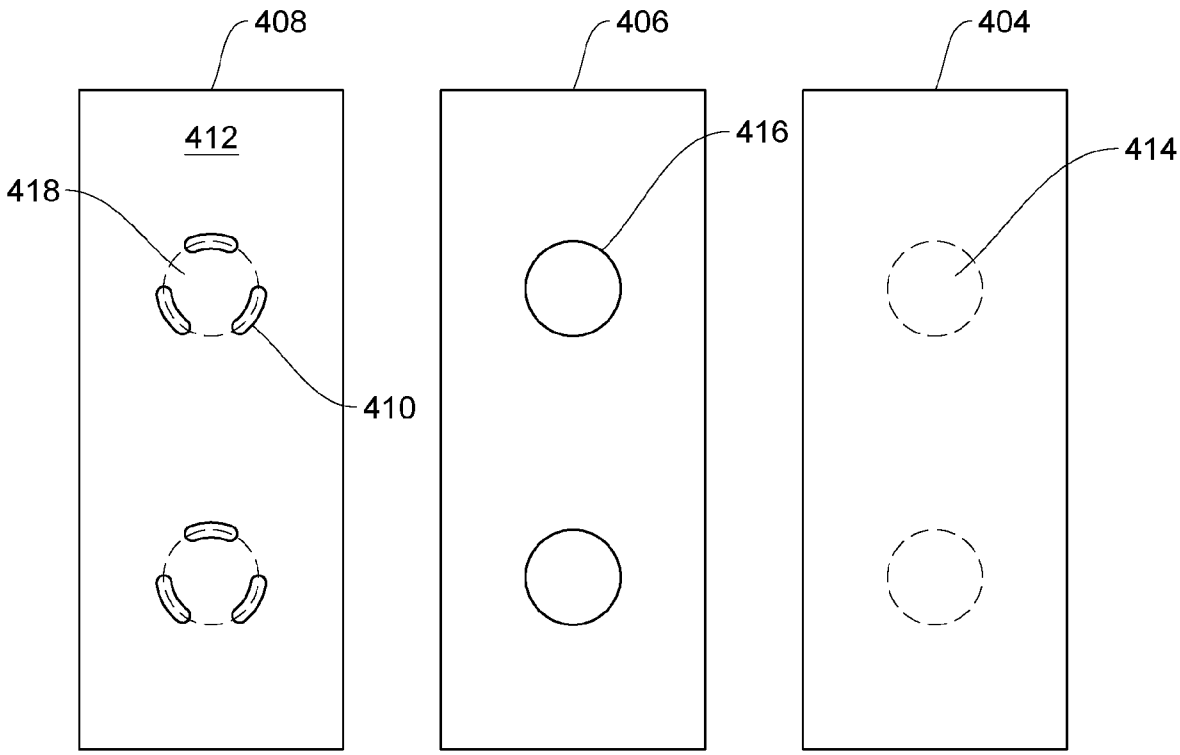


FIG. 4B

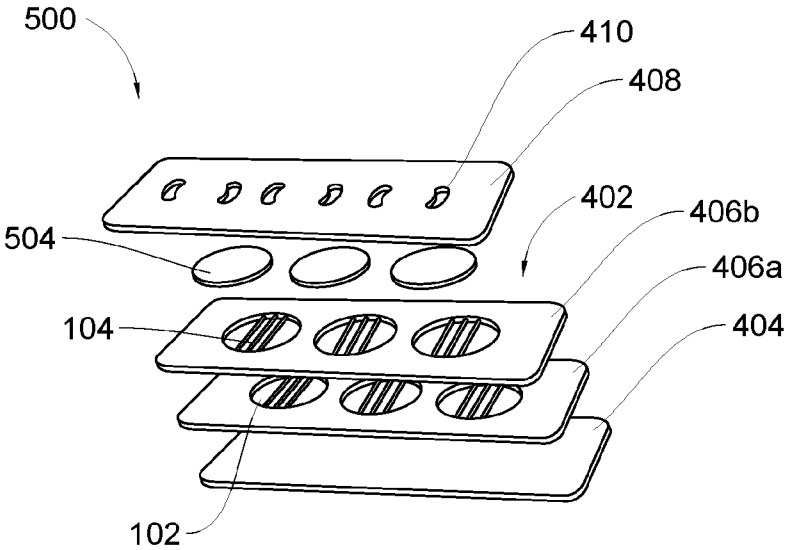

500
410
408
402
406b
504
406a
104
404
102

FIG. 5A

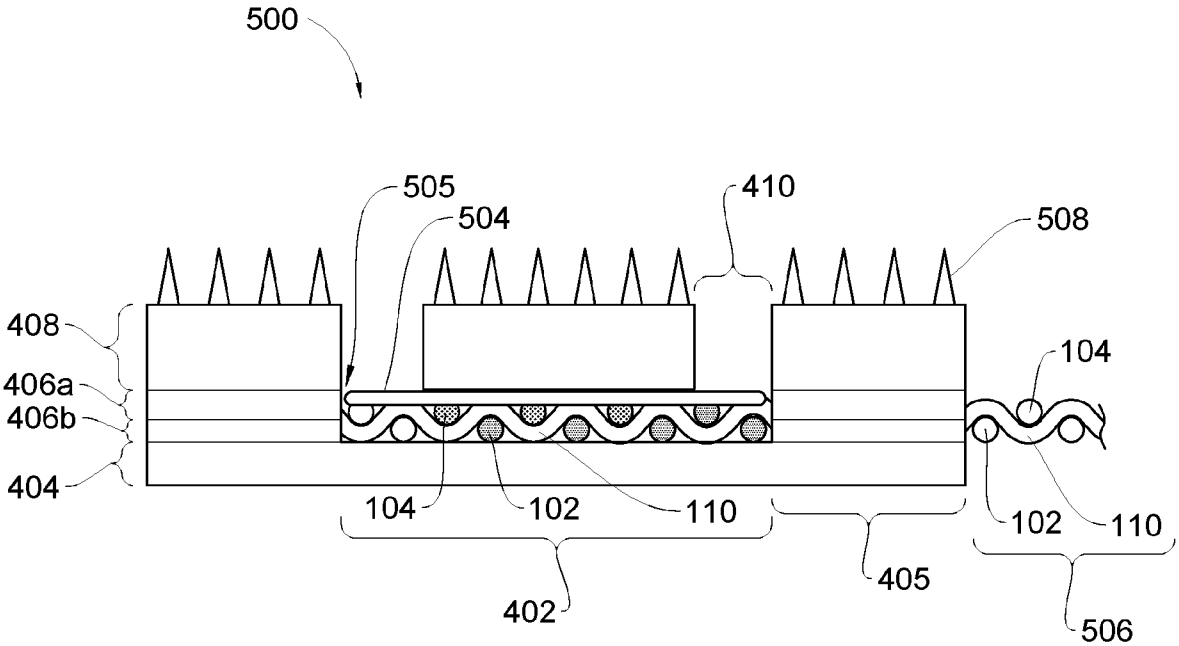

500
505
504
410
508
408
406a
406b
404
104
104
102
110
102
110
405
402
506

FIG. 5B

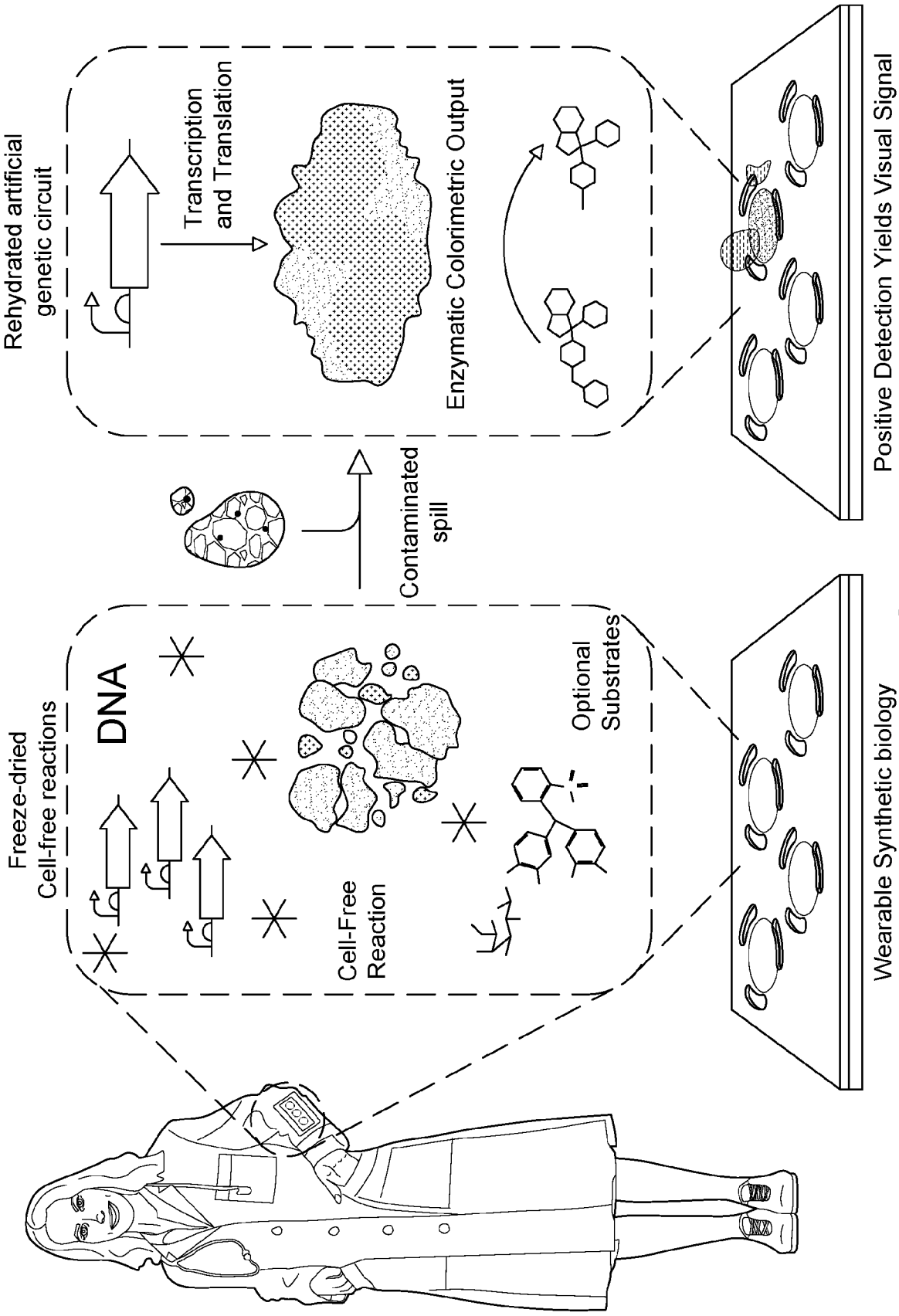

Freeze-dried
Cell-free reactions
DNA
Cell-Free
Reaction
Optional
Substrates
Contaminated
spill
Rehydrated artificial
genetic circuit
Transcription
and Translation
Enzymatic Colorimetric Output
Wearable Synthetic biology
Positive Detection Yields Visual Signal

FIG. 6A

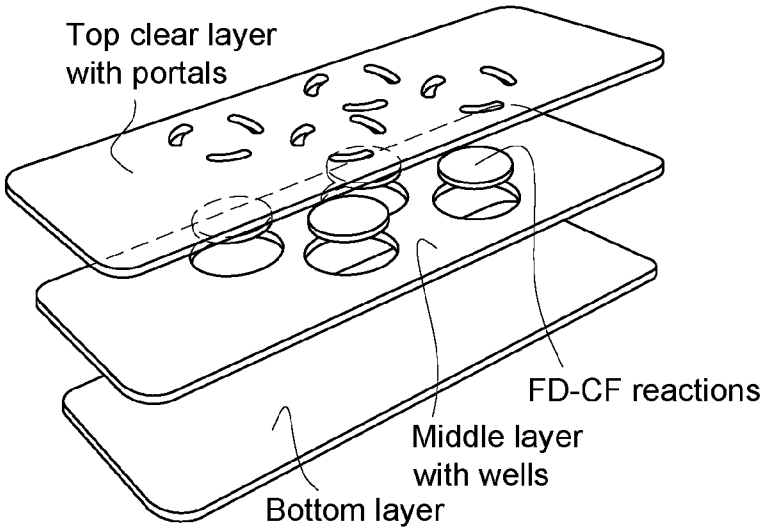


FIG. 6B

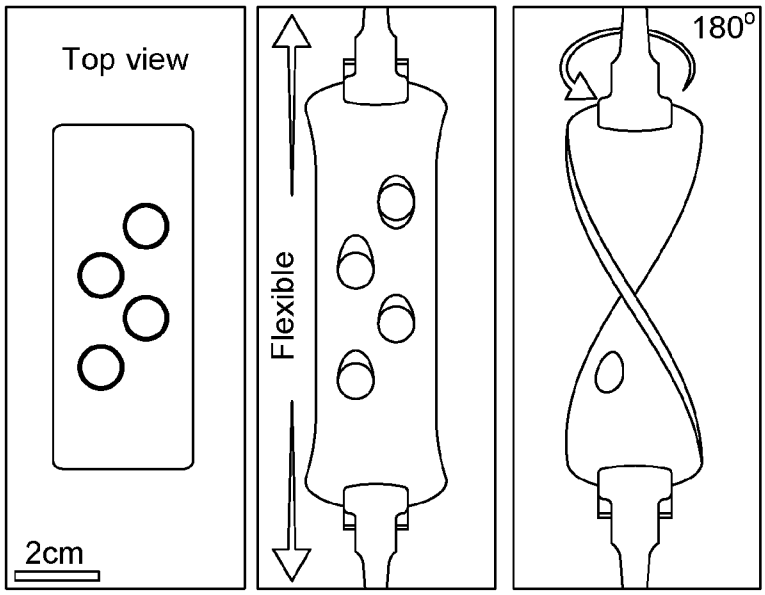


FIG. 6C

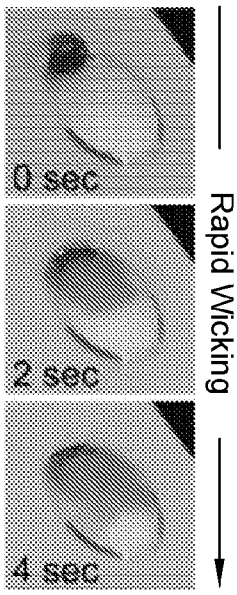


FIG. 6D

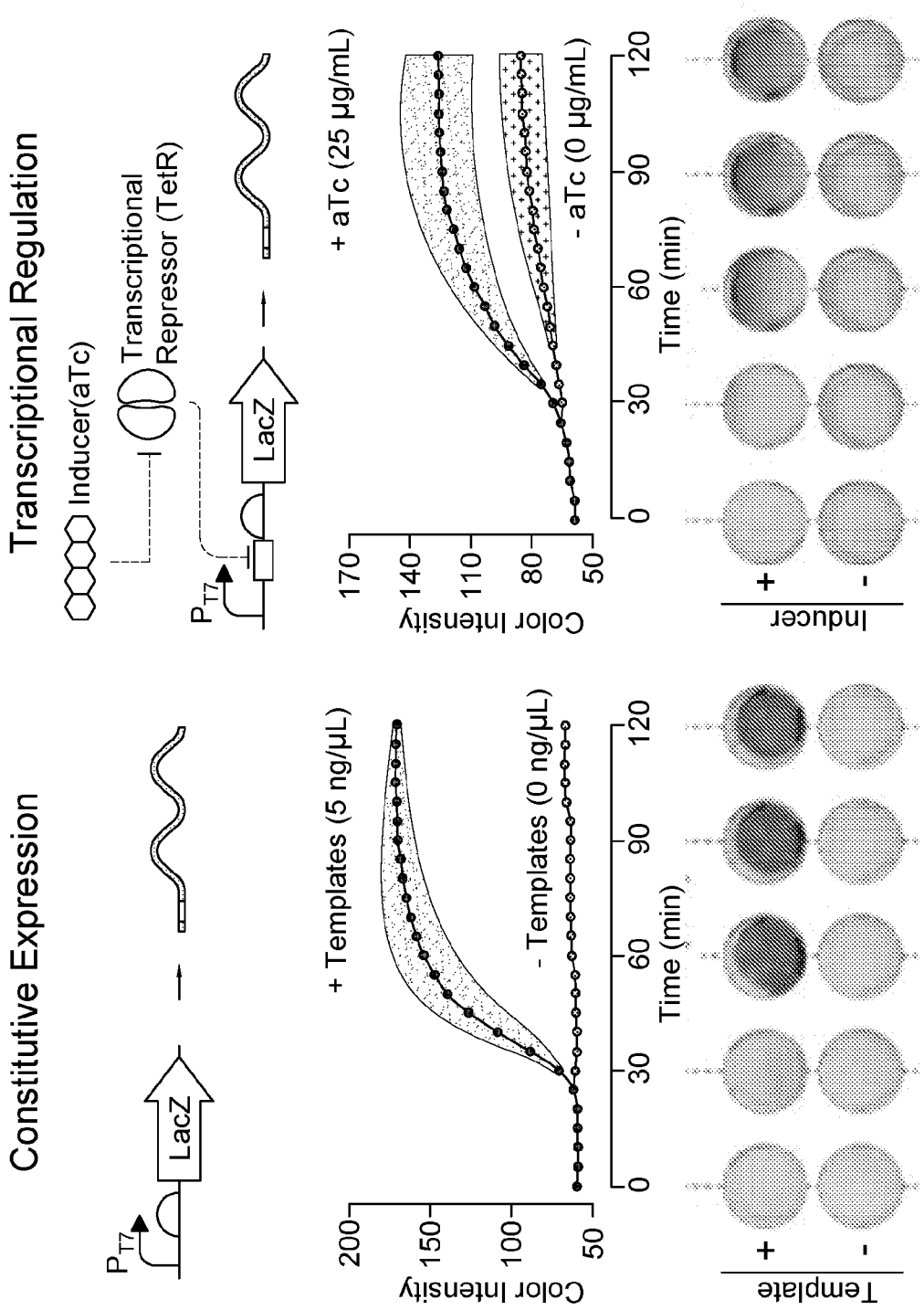

Constitutive Expression
P_{T7}
LacZ
+ Templates (5 ng/μL)
- Templates (0 ng/μL)
Color Intensity
200
150
100
50
0
30
60
90
120
Time (min)
Template
+
-
Transcriptional Regulation
Inducer(aTc)
Transcriptional Repressor (TetR)
P_{T7}
LacZ
+ aTc (25 μg/mL)
- aTc (0 μg/mL)
Color Intensity
170
140
110
80
50
0
30
60
90
120
Time (min)
Inducer
+
-

FIG. 6E

FIG. 6F

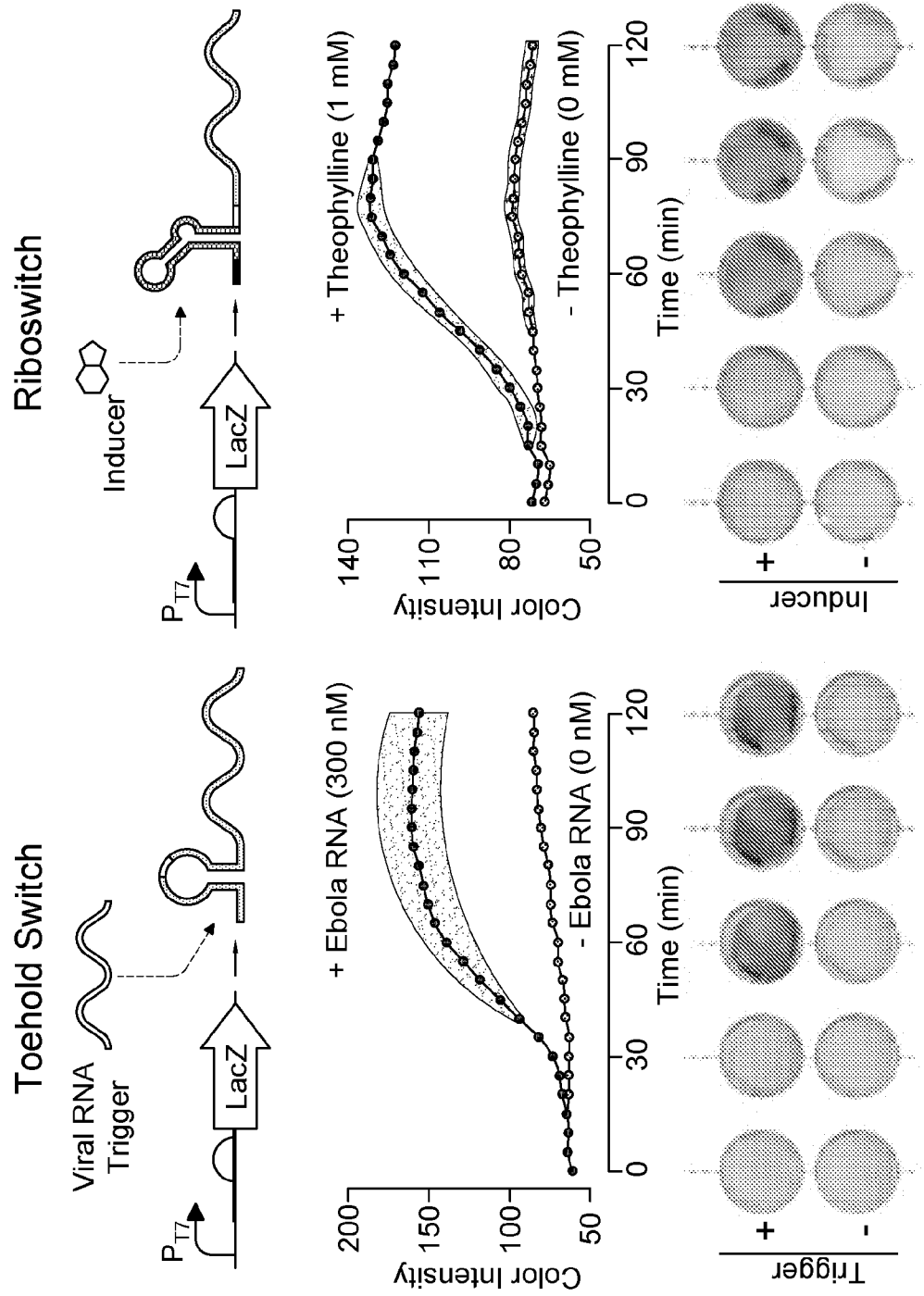

Toehold Switch
Viral RNA Trigger
LacZ
P_{T7}
+ Ebola RNA (300 nM)
- Ebola RNA (0 nM)
Color Intensity
200
150
100
50
0
30
60
90
120
Time (min)
Trigger
+
-

FIG. 6G

Riboswitch
Inducer
LacZ
P_{T7}
+ Theophylline (1 mM)
- Theophylline (0 mM)
Color Intensity
140
110
80
50
0
30
60
90
120
Time (min)
Inducer
+
-

FIG. 6H

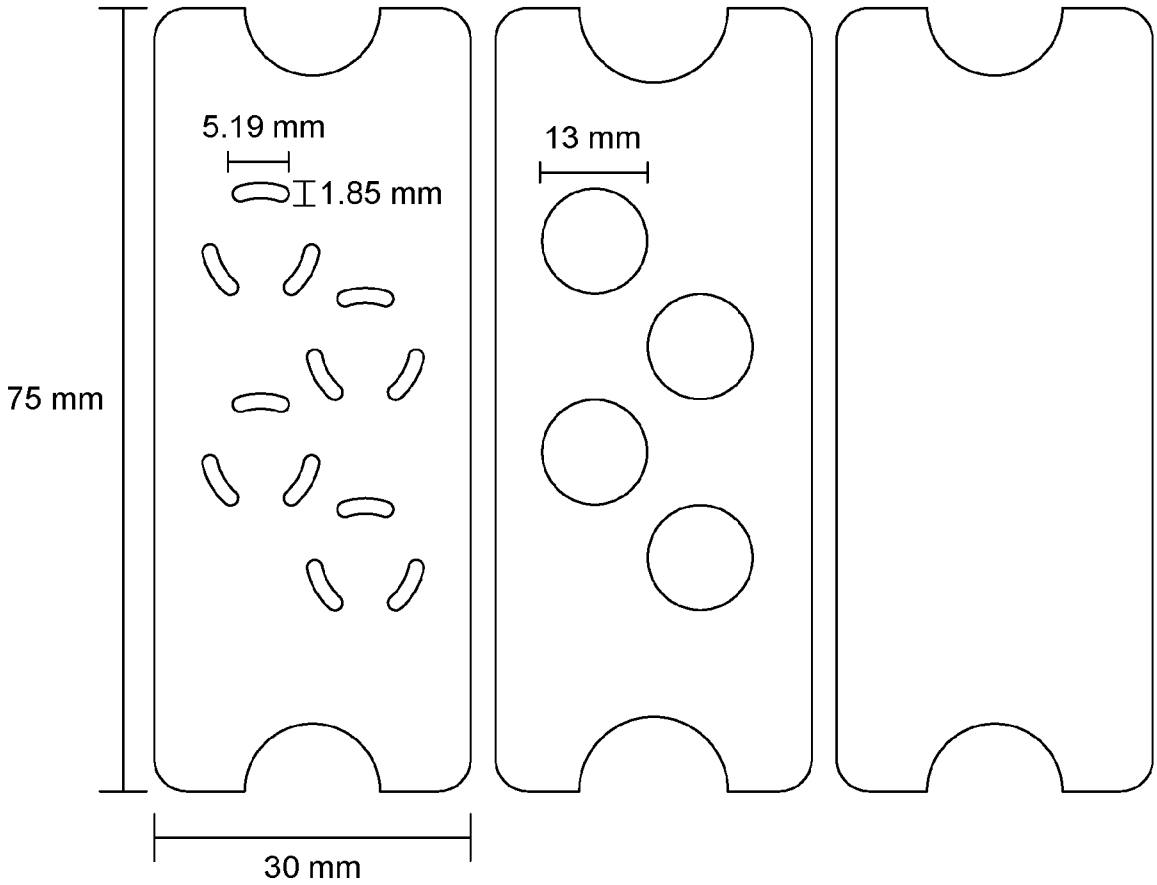


FIG. 7A

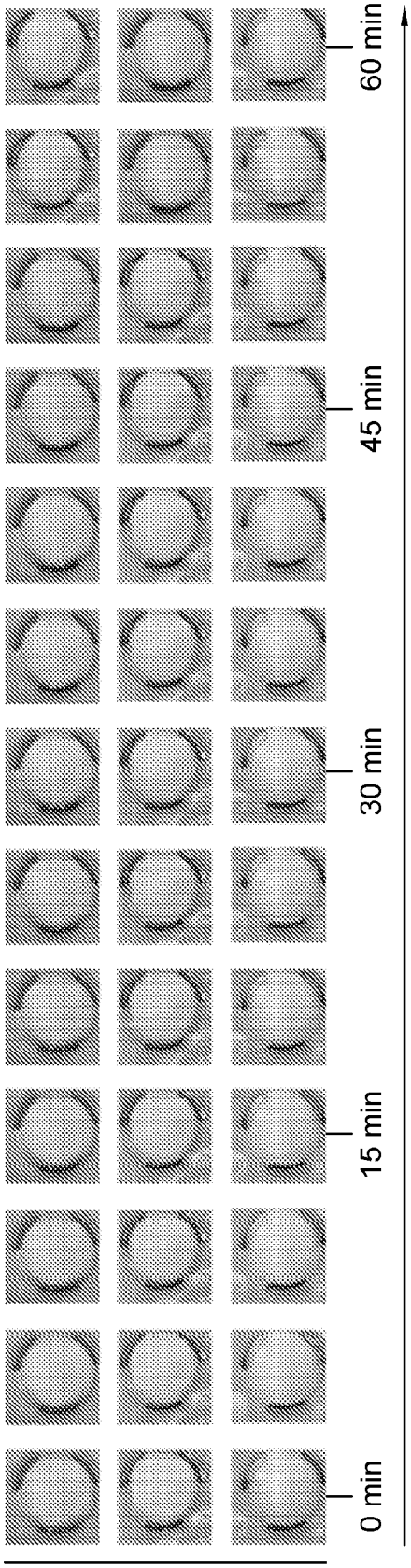

No Plasmid Control
0 min
15 min
30 min
45 min
60 min
Time

FIG. 7B

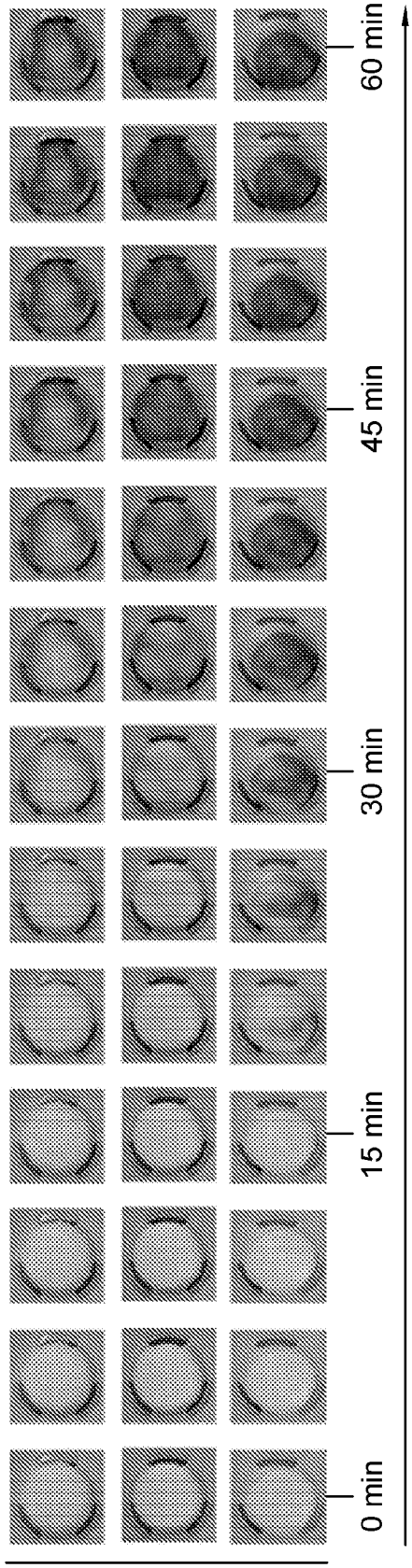

5 ng/μL pJL1-LacZ
0 min
15 min
30 min
45 min
60 min
Time

FIG. 7C

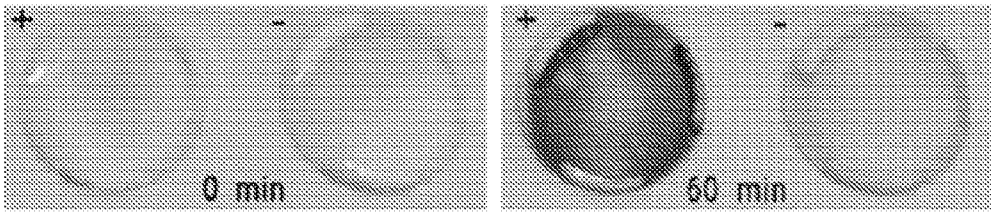


FIG. 8A

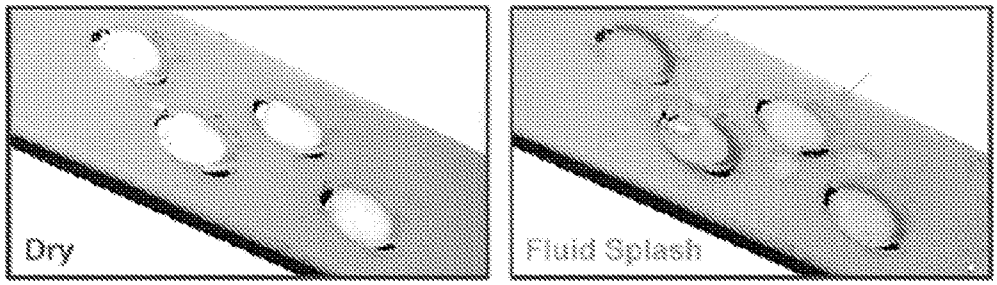


FIG. 8B

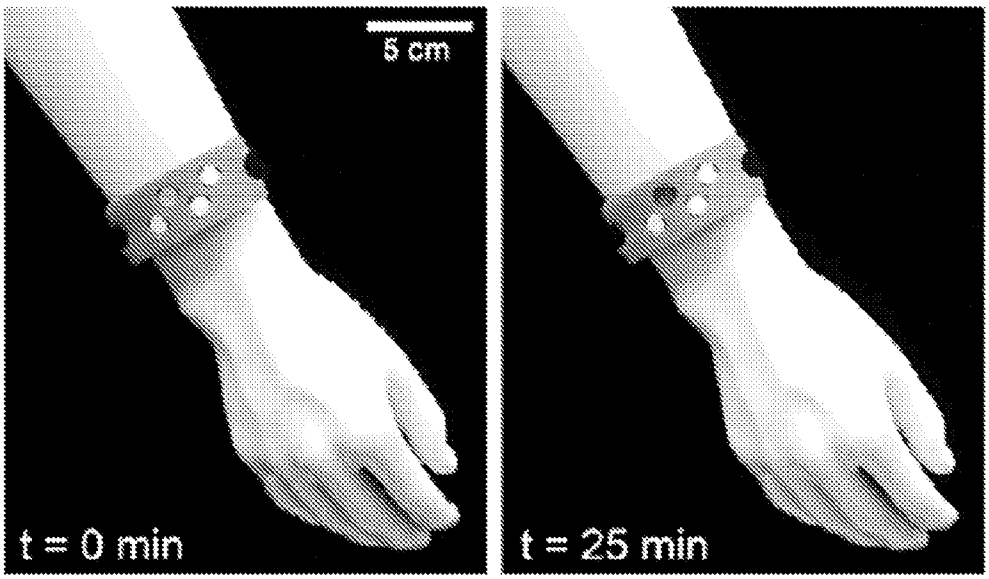


FIG. 8C

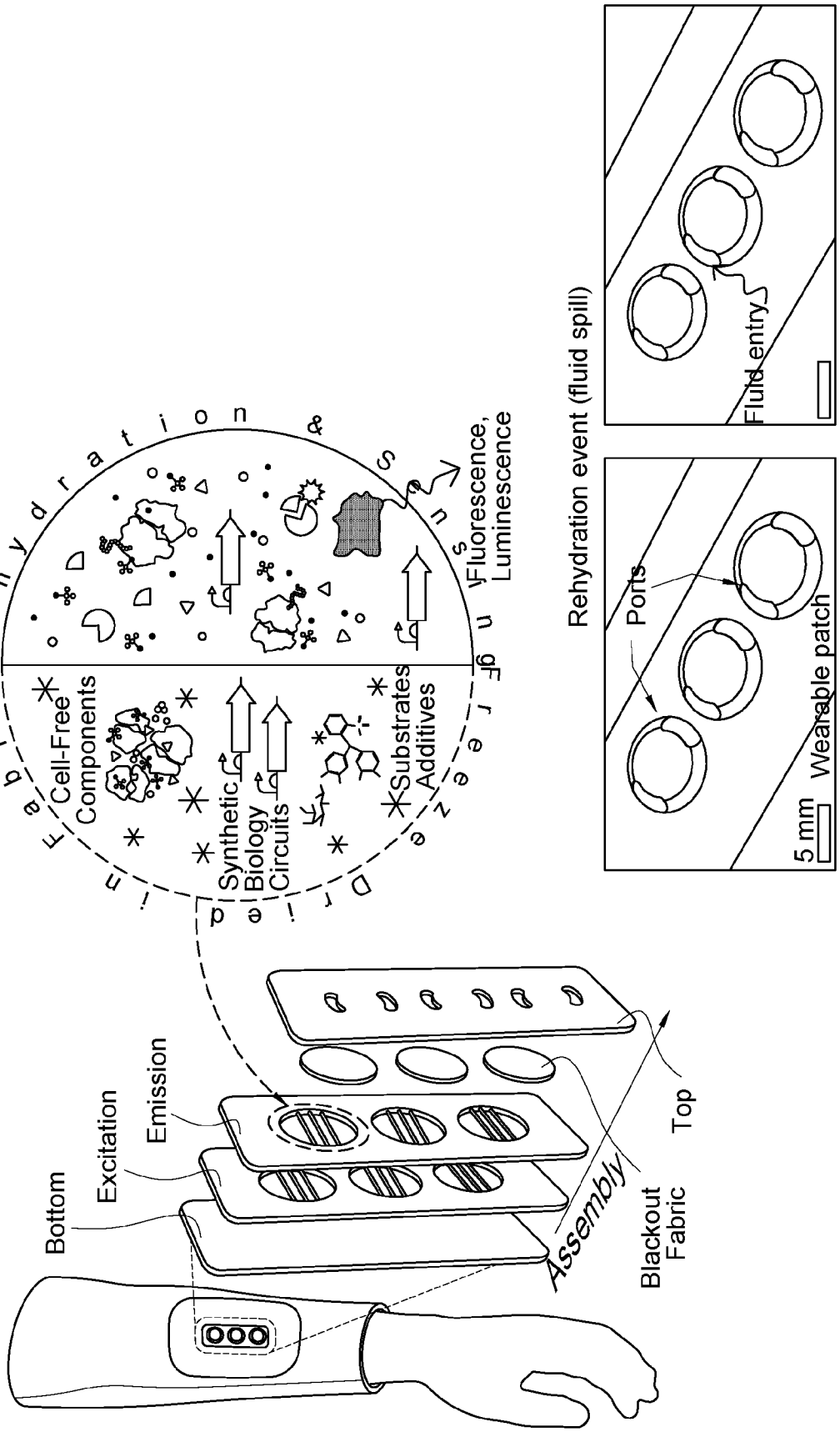

Lyophilized Cell-Free Components
Rehydration & Synthetic Biology Circuits
Substrates Additives
Fluorescence, Luminescence
Sensing
Rehydration event (fluid spill)
Ports
5 mm
Wearable patch
Fluid entry
Bottom
Excitation
Emission
Assembly
Blackout Fabric
Top

FIG. 9A

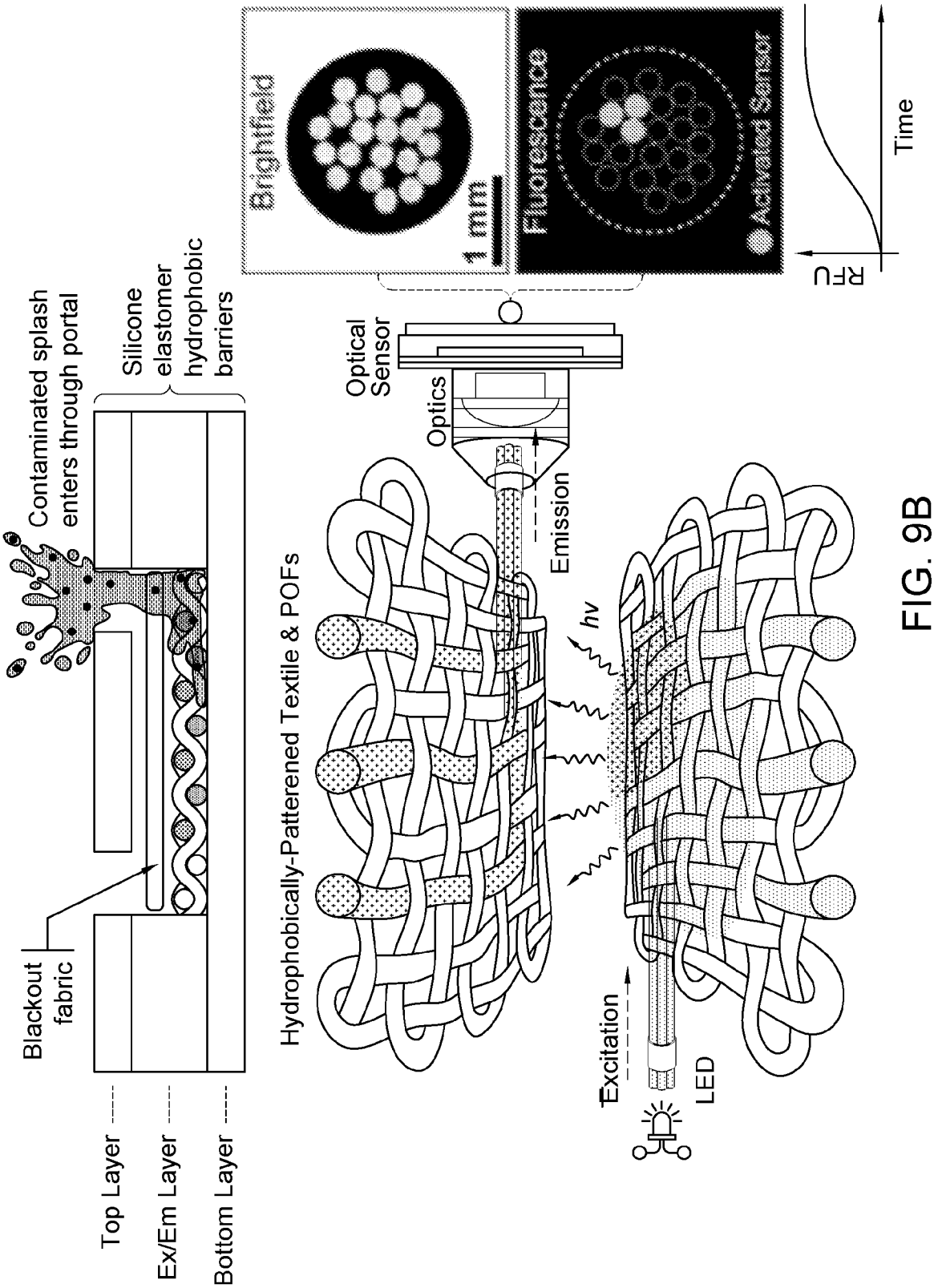

Contaminated splash enters through portal
Silicone elastomer hydrophobic barriers
Blackout fabric
Top Layer
Ex/Em Layer
Bottom Layer
Hydrophobically-Patterened Textile & POFs
Optical Sensor
Optics
Emission
hv
Excitation
LED
Brightfield
1 mm
Fluorescence
Activated Sensor
RFU
Time

FIG. 9B

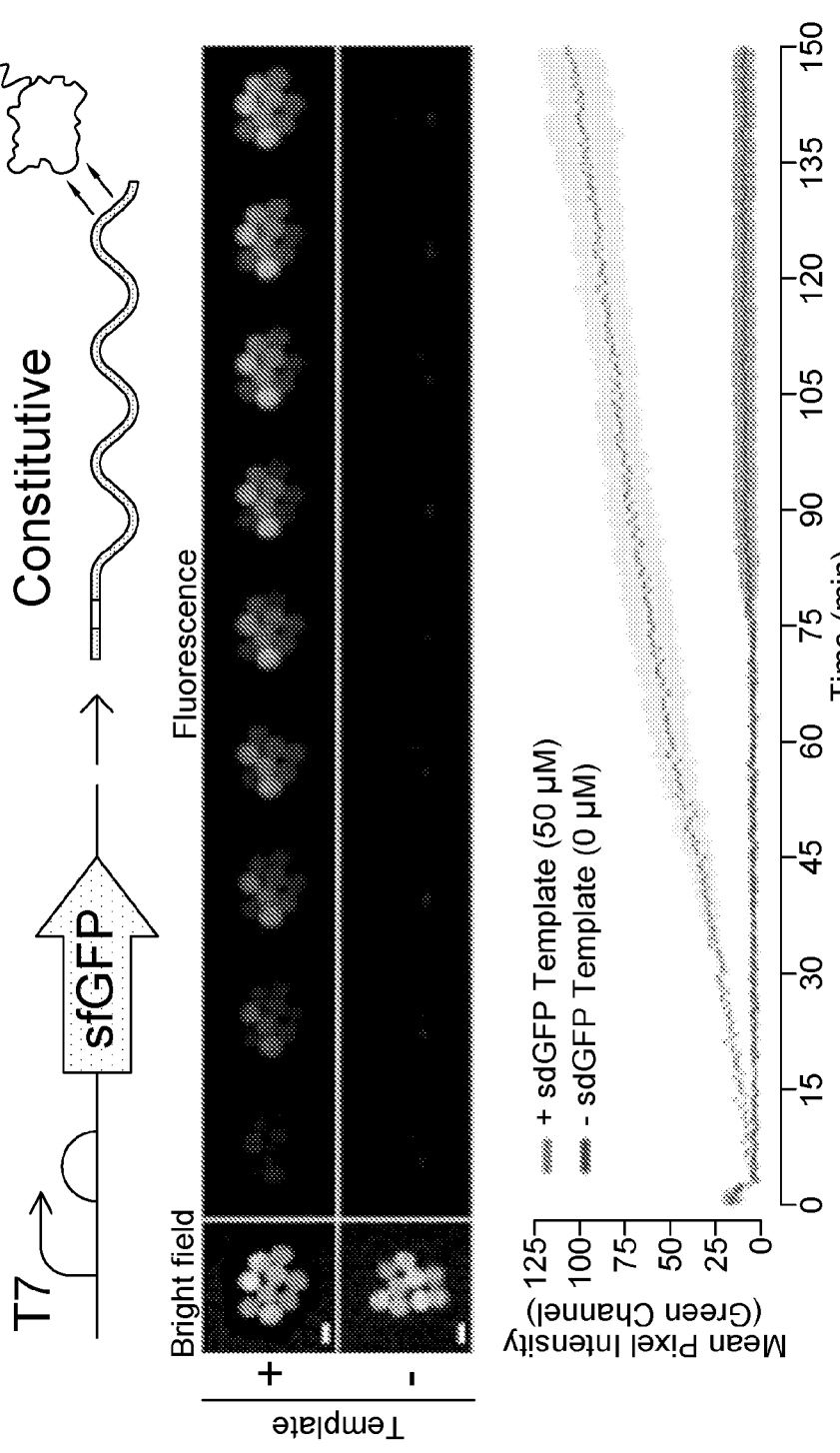

hv
Constitutive
T7
sfGFP
Fluorescence
Bright field
Template
+
-
Mean Pixel Intensity (Green Channel)
125
100
75
50
25
0
+ sdGFP Template (50 µM)
- sdGFP Template (0 µM)
0
15
30
45
60
75
90
105
120
135
150
Time (min)

FIG. 9C

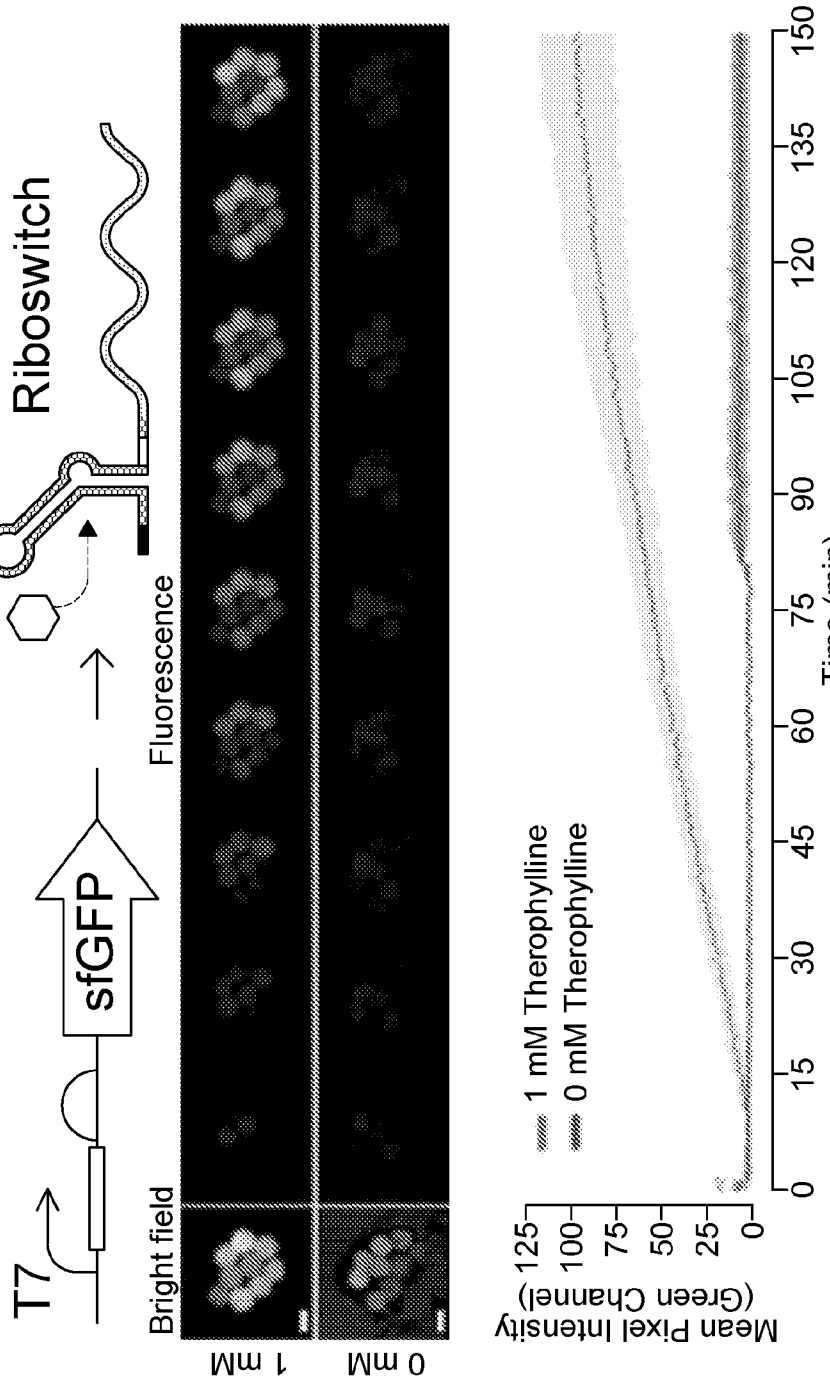

T7
sfGFP
Riboswitch
Fluorescence
Bright field
Theophylline
0 mM
1 mM
Mean Pixel Intensity (Green Channel)
0
25
50
75
100
125
1 mM Therophylline
0 mM Therophylline
0
15
30
45
60
75
90
105
120
135
150
Time (min)

FIG. 9D

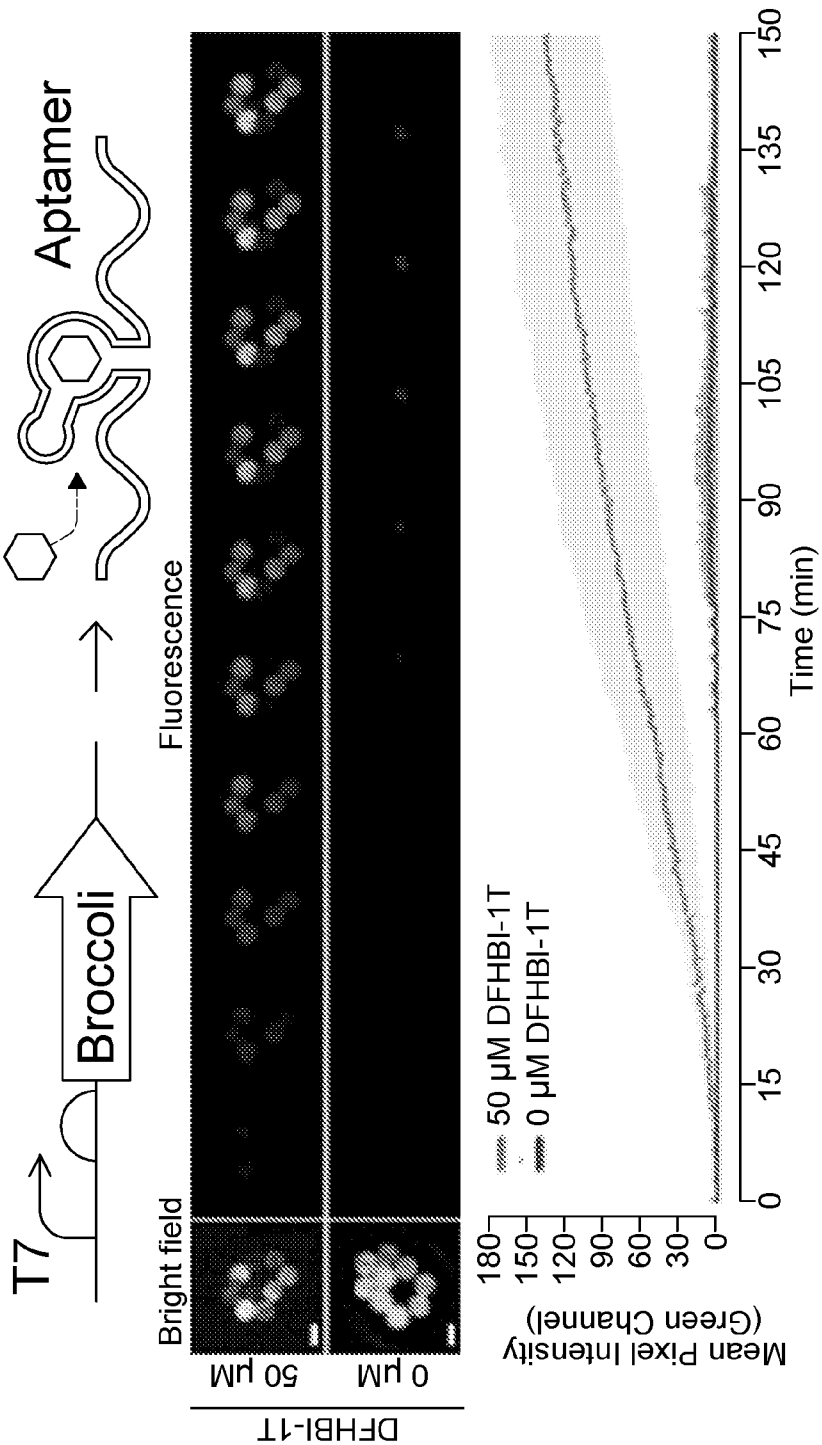

T7
Broccoli
Aptamer
Bright field
Fluorescence
DFHBI-1T
50 µM
0 µM
50 µM DFHBI-1T
0 µM DFHBI-1T
Mean Pixel Intensity (Green Channel)
180
150
120
90
60
30
0
0
15
30
45
60
75
90
105
120
135
150
Time (min)

FIG. 9E

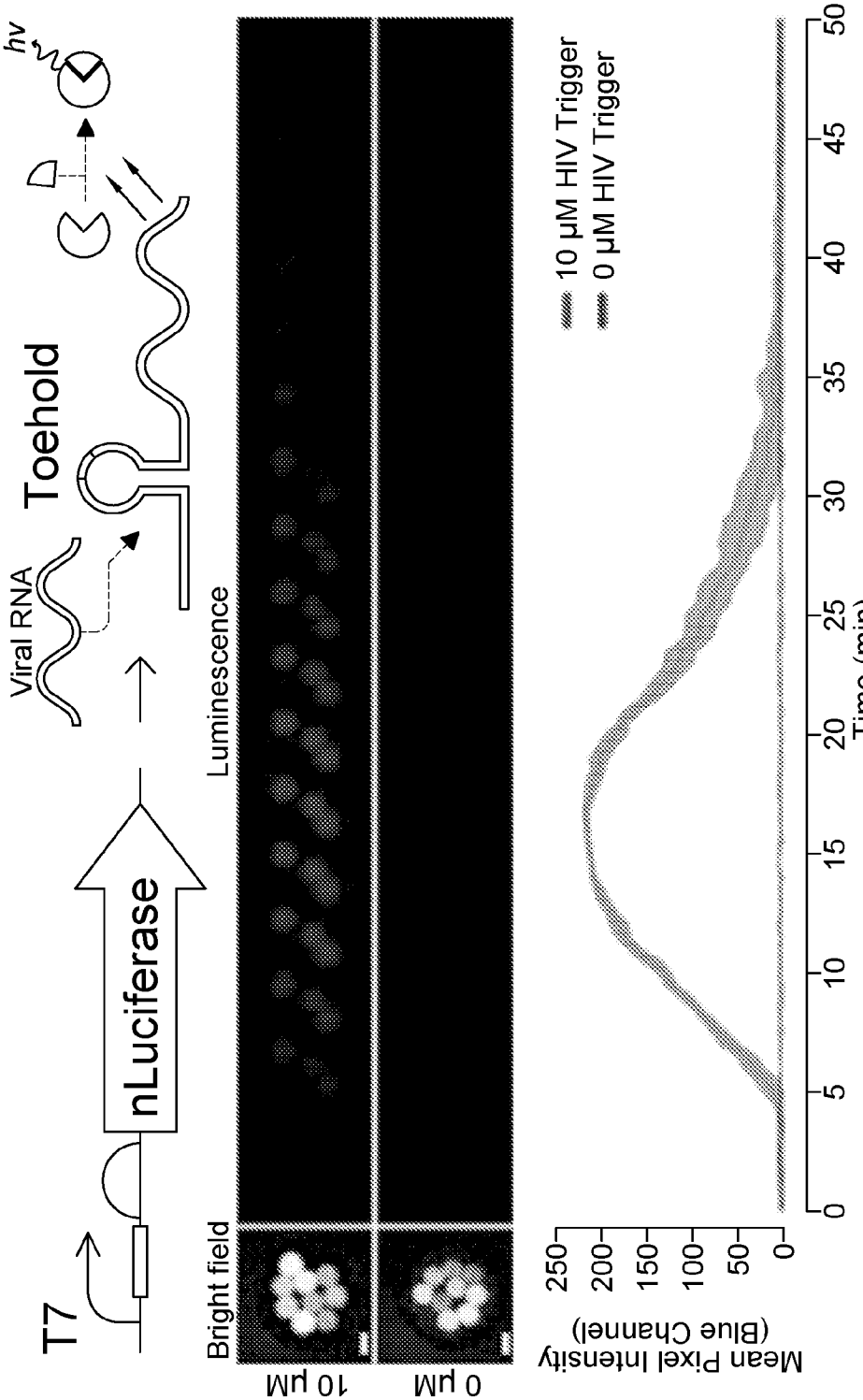

T7
nLuciferase
Viral RNA
Toehold
hv
Theophylline
10 μM
0 μM
Bright field
Luminescence
10 μM HIV Trigger
0 μM HIV Trigger
Mean Pixel Intensity (Blue Channel)
250
200
150
100
50
0
0
5
10
15
20
25
30
35
40
45
50
Time (min)

FIG. 9F

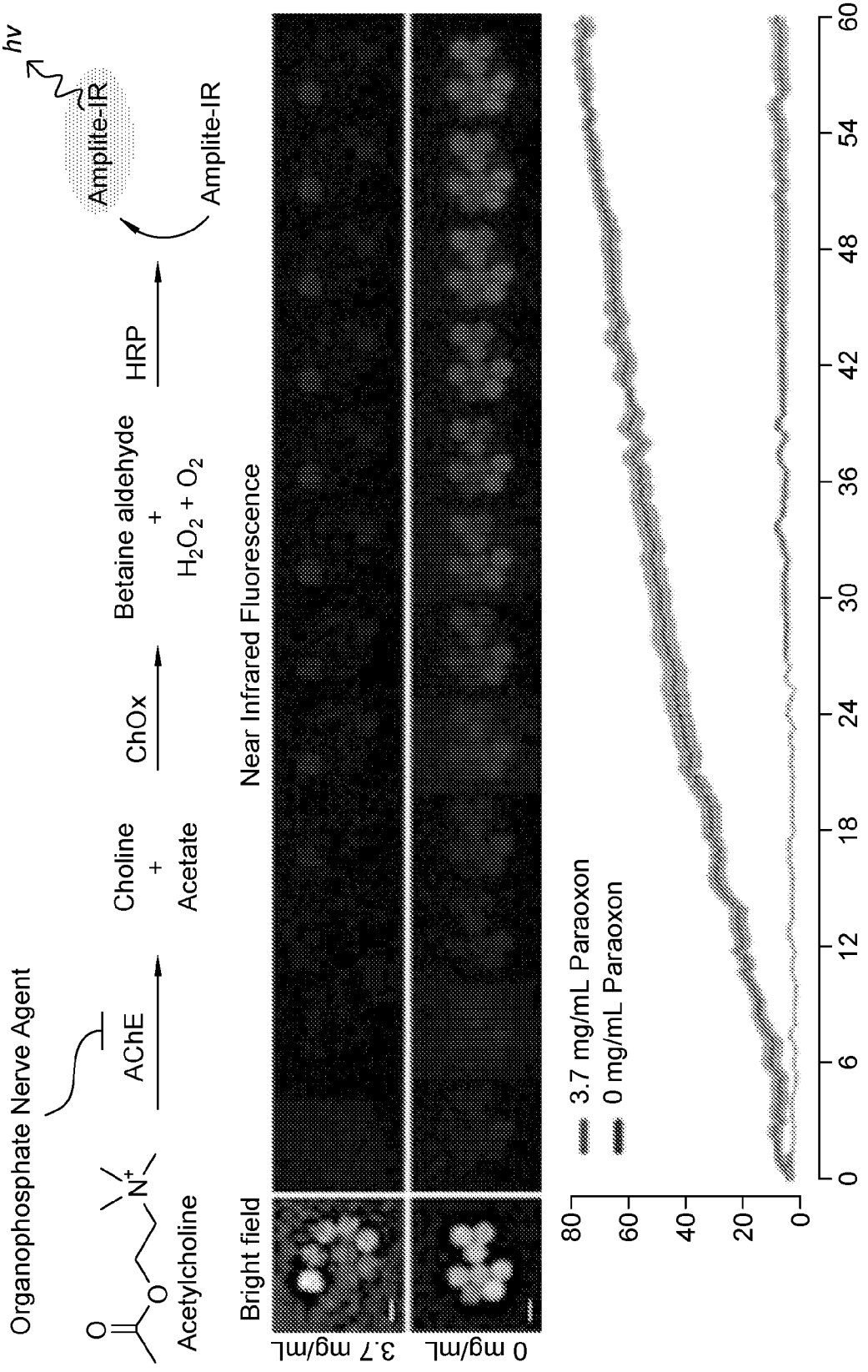

Organophosphate Nerve Agent
Acetylcholine
AChE
Choline
+
Acetate
ChOx
Betaine aldehyde
+
$H_2O_2 + O_2$
HRP
Amplite-IR
hν
Amplite-IR
Bright field
Near Infrared Fluorescence
3.7 mg/mL
0 mg/mL
Paraoxon-ethyl
3.7 mg/mL Paraoxon
0 mg/mL Paraoxon
80
60
40
20
0
0
6
12
18
24
30
36
42
48
54
60

FIG. 9G

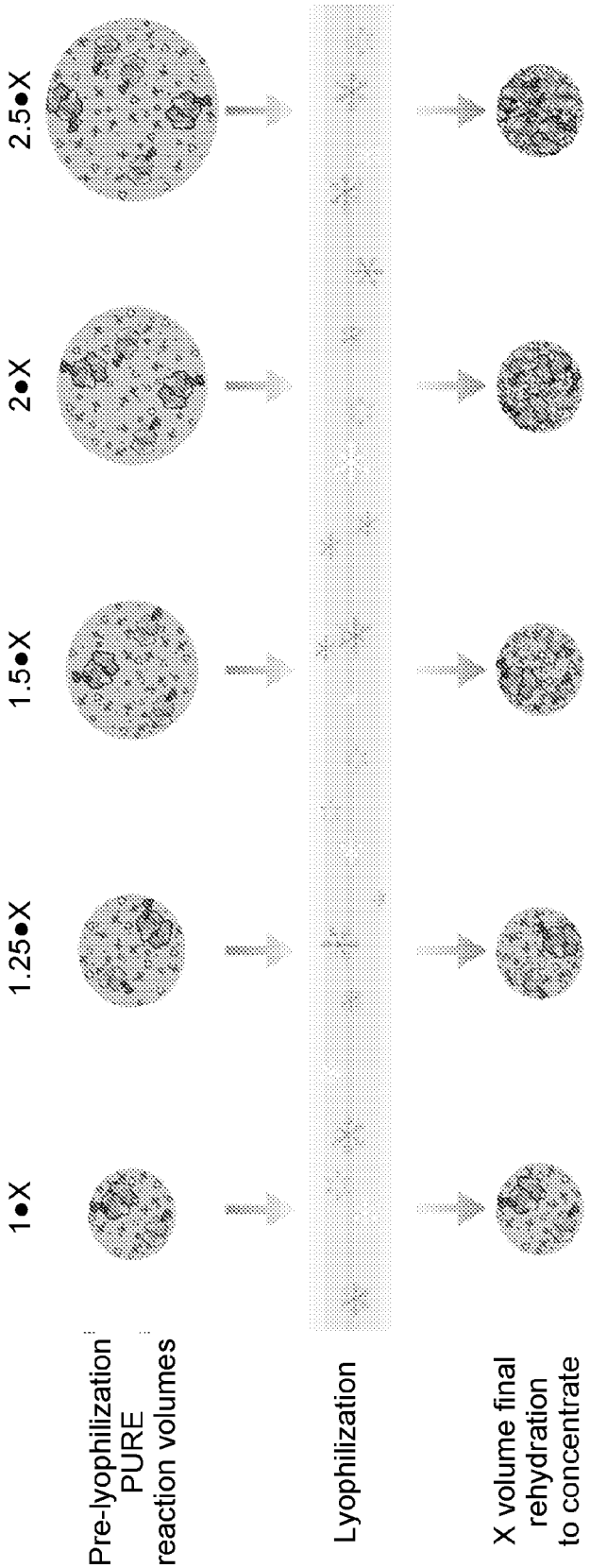

1•X
1.25•X
1.5•X
2•X
2.5•X
Pre-lyophilization PURE reaction volumes
Lyophilization
X volume final rehydration to concentrate

FIG. 10A

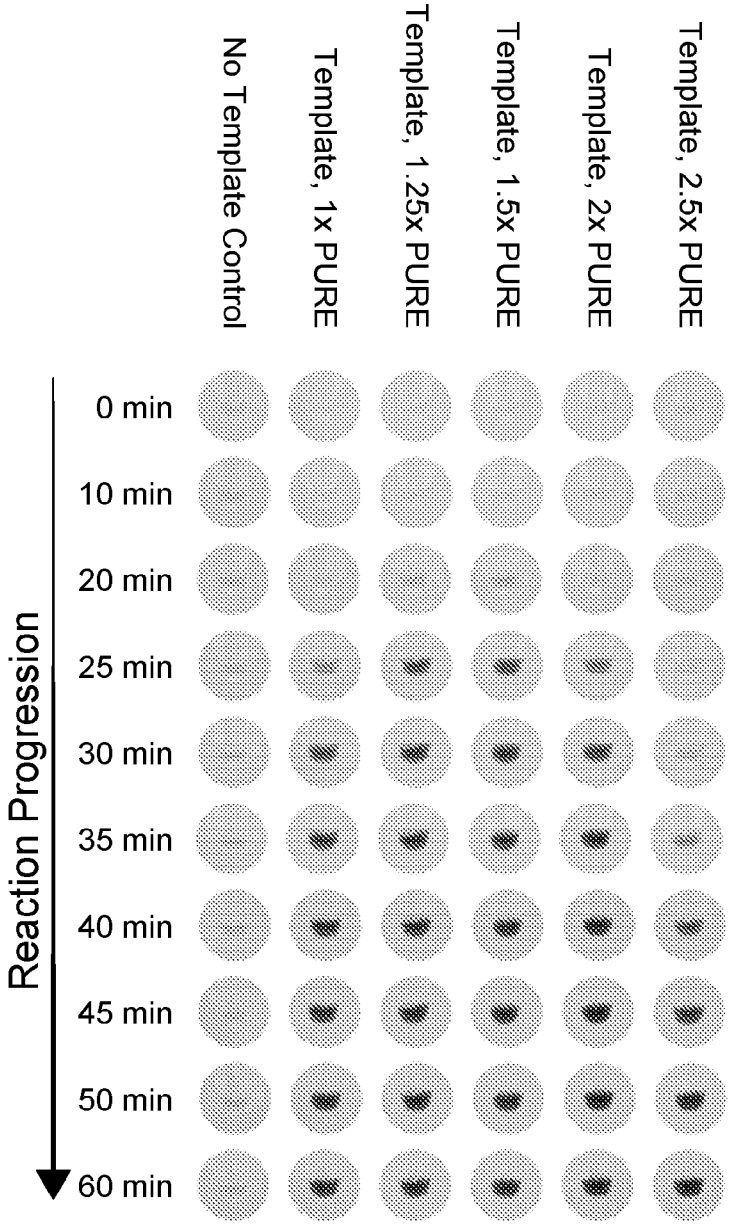

No Template Control
Template, 1x PURE
Template, 1.25x PURE
Template, 1.5x PURE
Template, 2x PURE
Template, 2.5x PURE
Reaction Progression
0 min
10 min
20 min
25 min
30 min
35 min
40 min
45 min
50 min
60 min

FIG. 10B

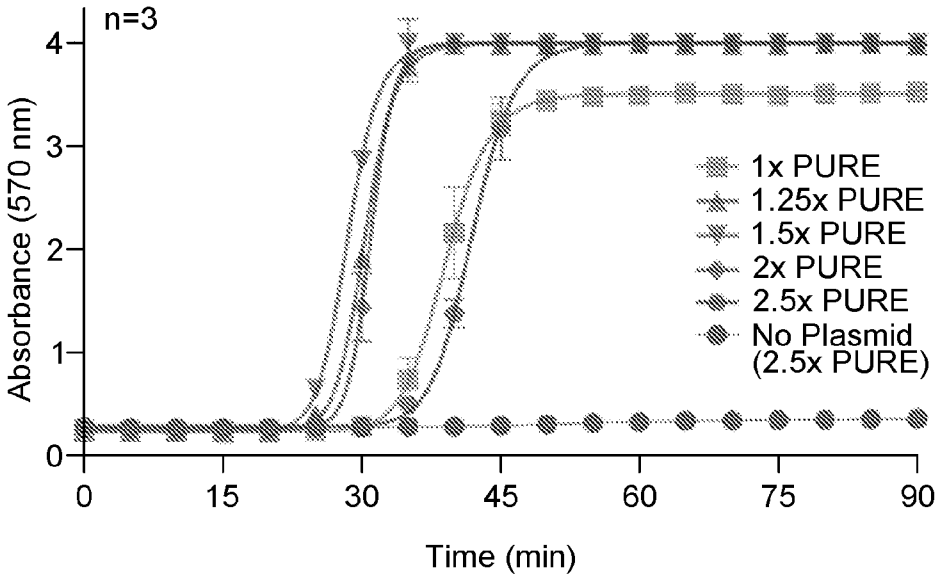


FIG. 10C

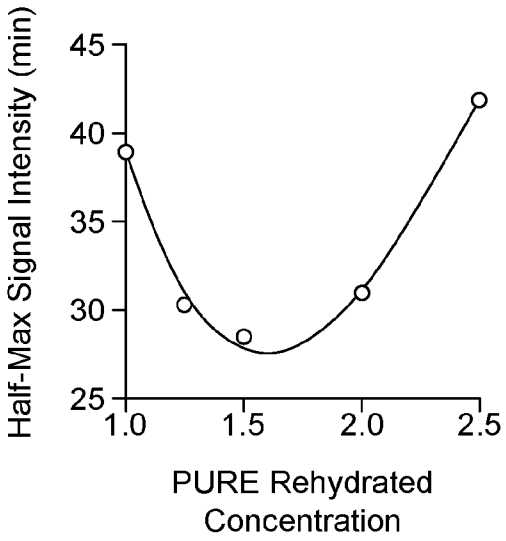


FIG. 10D

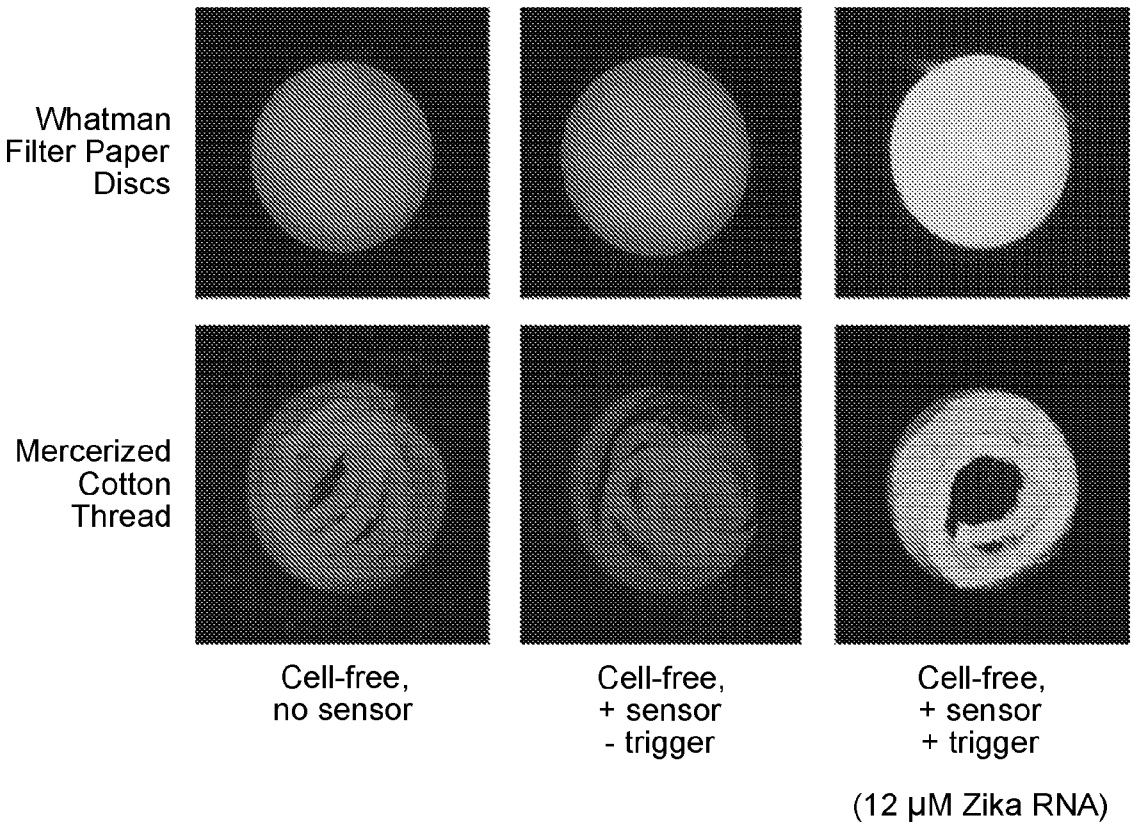

Whatman Filter Paper Discs
Mercerized Cotton Thread
Cell-free, no sensor
Cell-free, + sensor - trigger
Cell-free, + sensor + trigger
(12 μM Zika RNA)

FIG. 11

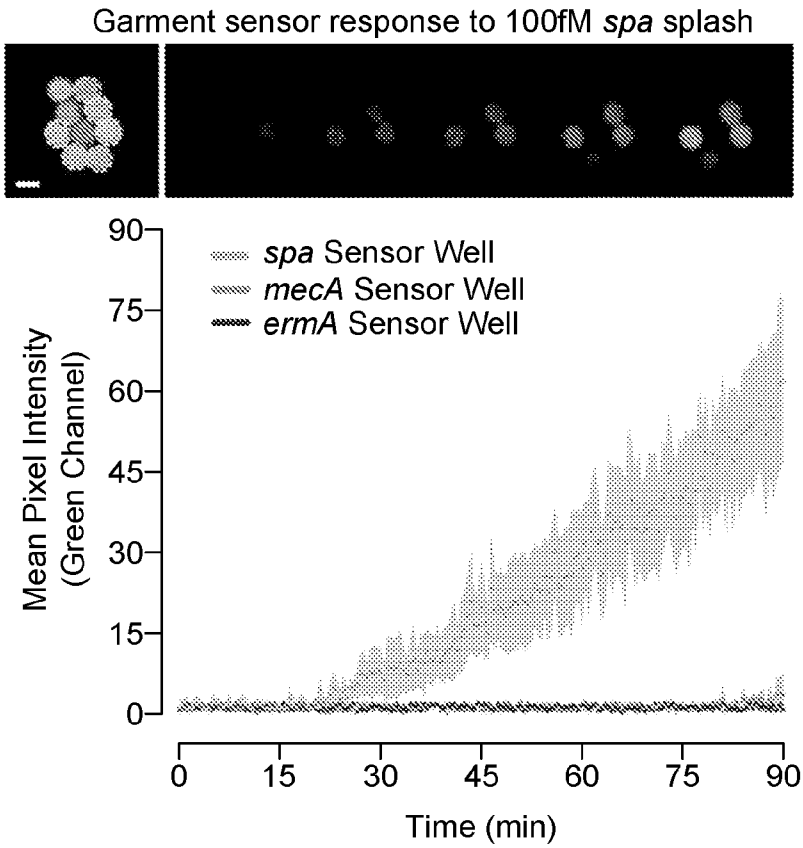

Garment sensor response to 100fM spa splash
spa Sensor Well
mecA Sensor Well
ermA Sensor Well
Mean Pixel Intensity (Green Channel)
0
15
30
45
60
75
90
Time (min)

FIG. 12

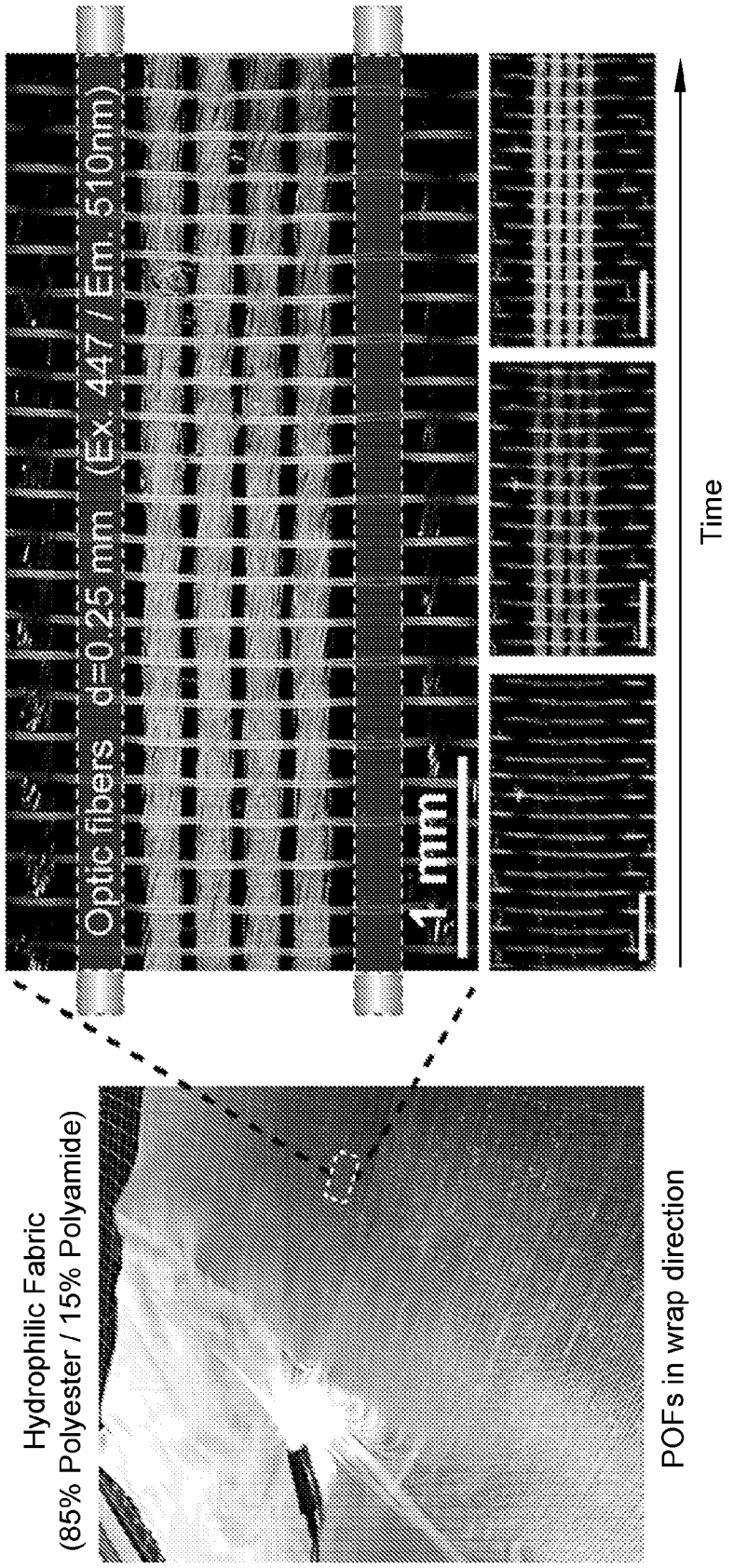

Optic fibers d=0.25 mm (Ex. 447 / Em. 510nm)
1 mm
Time
Hydrophilic Fabric
(85% Polyester / 15% Polyamide)
POFs in wrap direction

FIG. 13

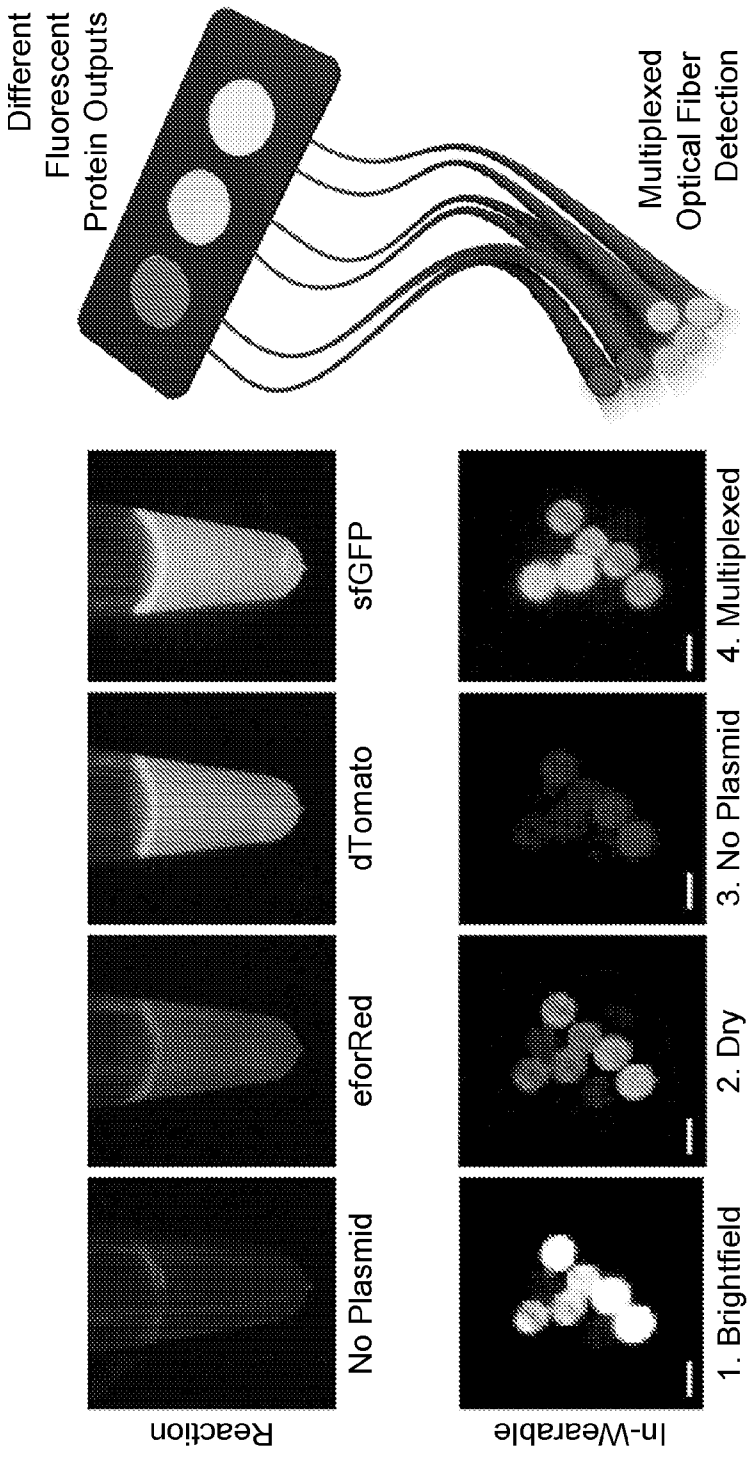

Different
Fluorescent
Protein Outputs
Multiplexed
Optical Fiber
Detection
Reaction
No Plasmid
eforRed
dTomato
sfGFP
In-Wearable
1. Brightfield
2. Dry
3. No Plasmid
4. Multiplexed

FIG. 14

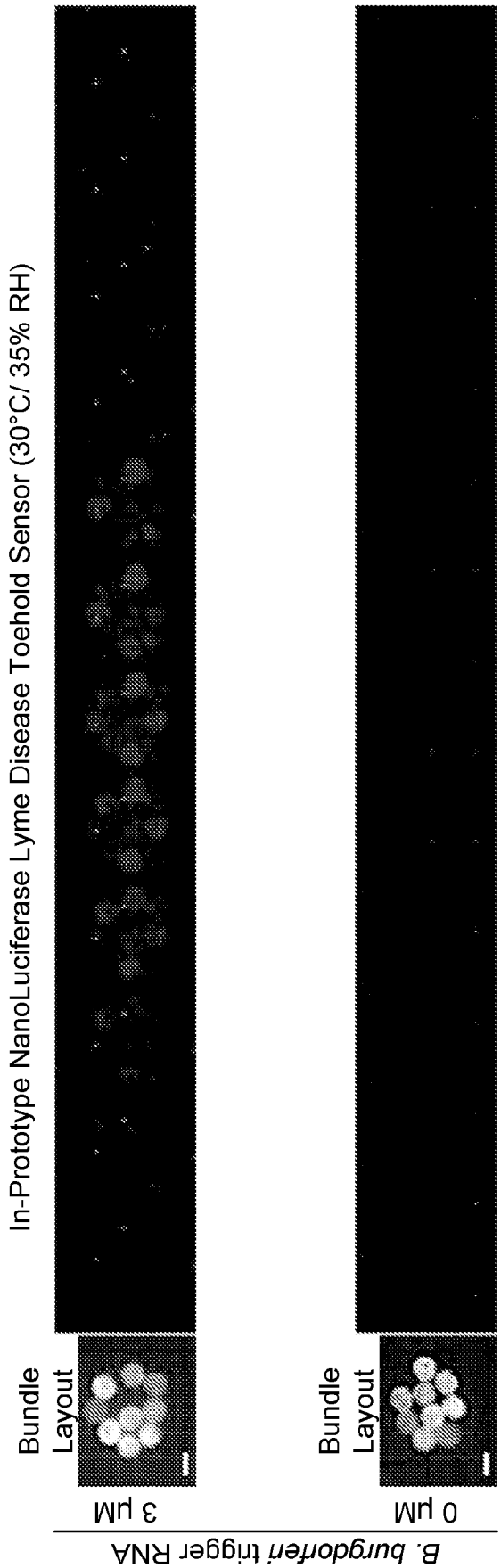

In-Prototype NanoLuciferase Lyme Disease Toehold Sensor (30°C/ 35% RH)
B. burgdorferi trigger RNA
3 μM
0 μM
Bundle Layout
Bundle Layout
3 μM Lyme Trigger
0 μM Lyme Trigger
Average Pixel Intensity in Sensor Bundle (Blue Channel)
0
50
100
150
200
250
300
0
5
10
15
20
25
30
35
Time (min)

FIG. 15A

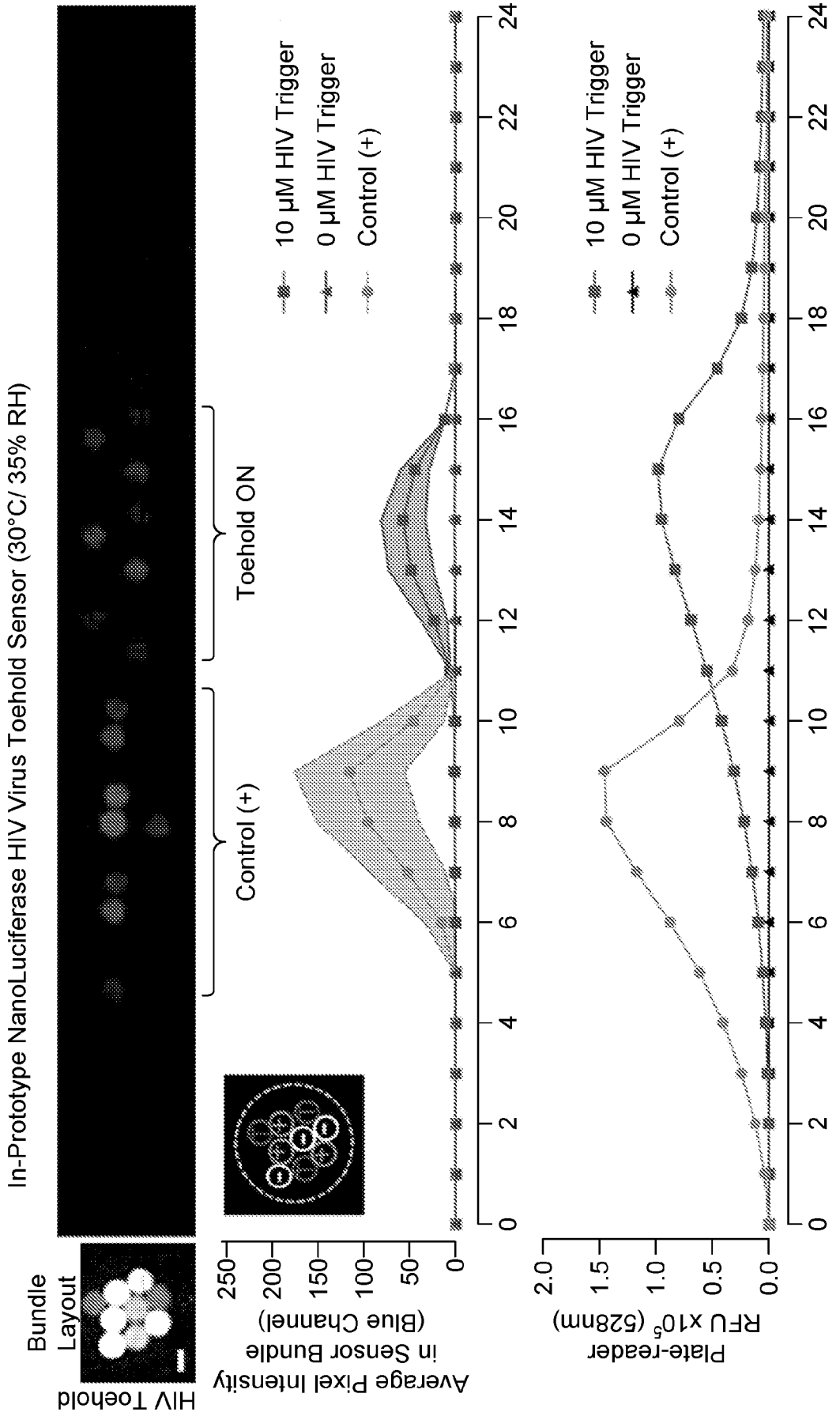

In-Prototype NanoLuciferase HIV Virus Toehold Sensor (30°C/ 35% RH)
HIV Toehold
Bundle Layout
Control (+)
Toehold ON
10 µM HIV Trigger
0 µM HIV Trigger
Control (+)
Average Pixel Intensity in Sensor Bundle (Blue Channel)
250
200
150
100
50
0
0
2
4
6
8
10
12
14
16
18
20
22
24
Plate-reader RFU x10^5 (528nm)
2.0
1.5
1.0
0.5
0.0
Time (min)

FIG. 15B

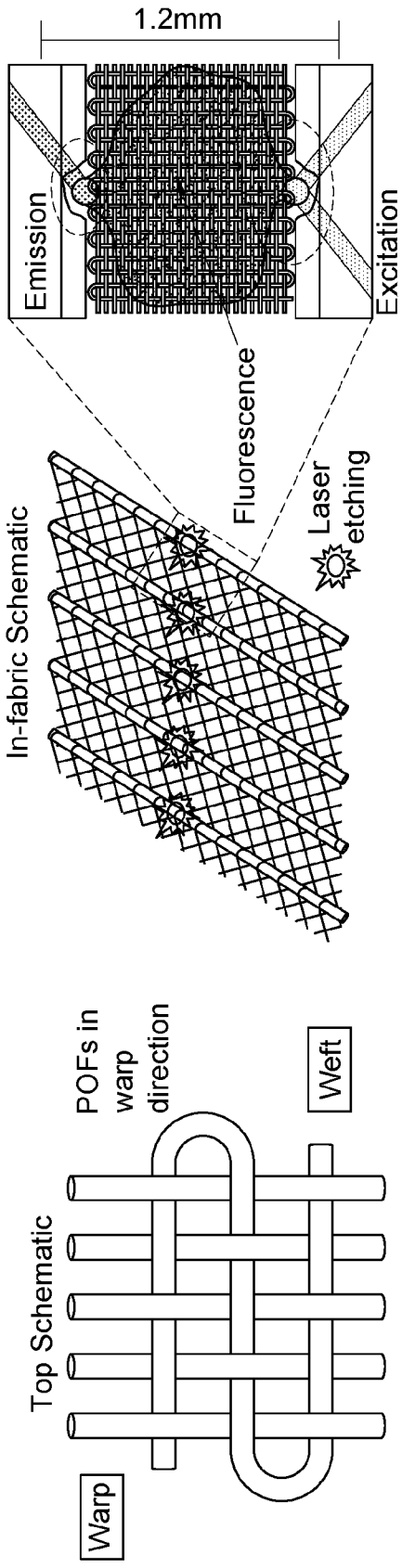

1.2mm
Emission
Excitation
Fluorescence
Laser etching
In-fabric Schematic
POFs in warp direction
Weft
Top Schematic
Warp

FIG. 16A

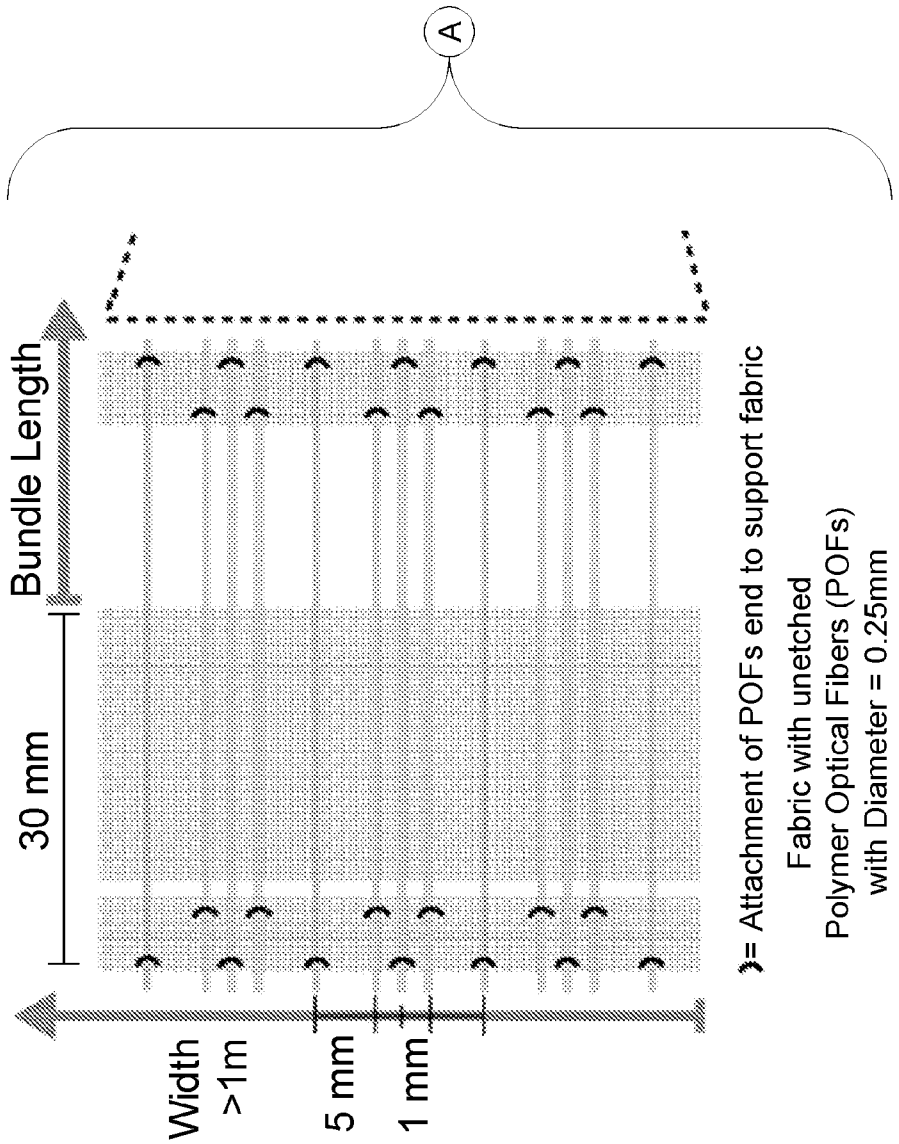

A
Bundle Length
30 mm
Width
>1m
5 mm
1 mm
= Attachment of POFs end to support fabric
Fabric with unetched
Polymer Optical Fibers (POFs)
with Diameter = 0.25mm

FIG. 16B

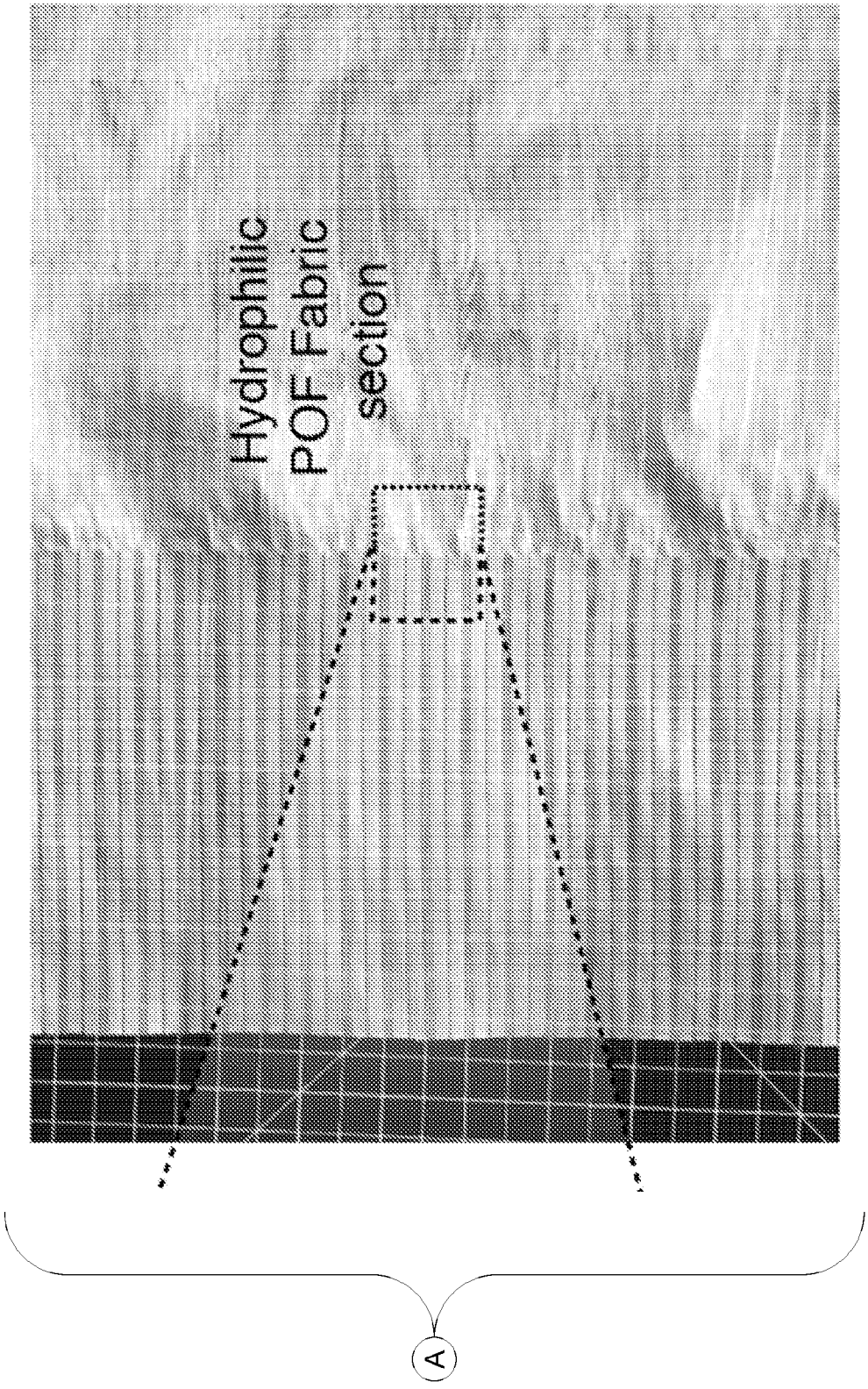


FIG. 16B (CONTINUATION)

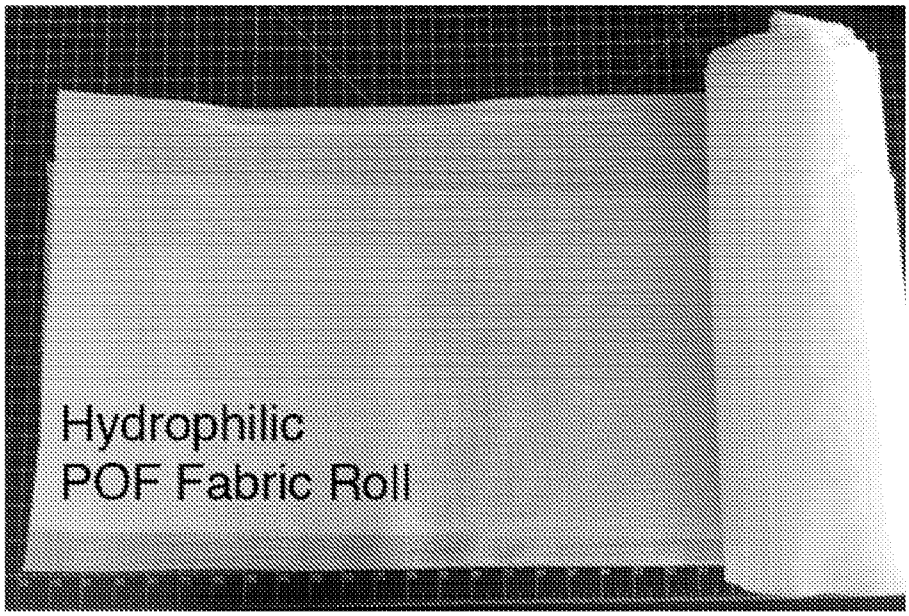


FIG. 16C

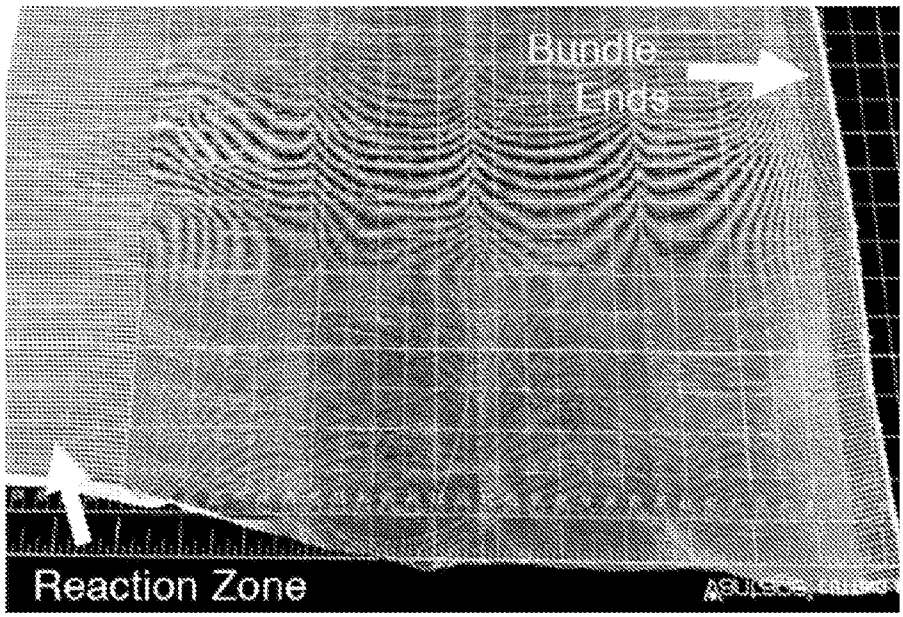


FIG. 16D

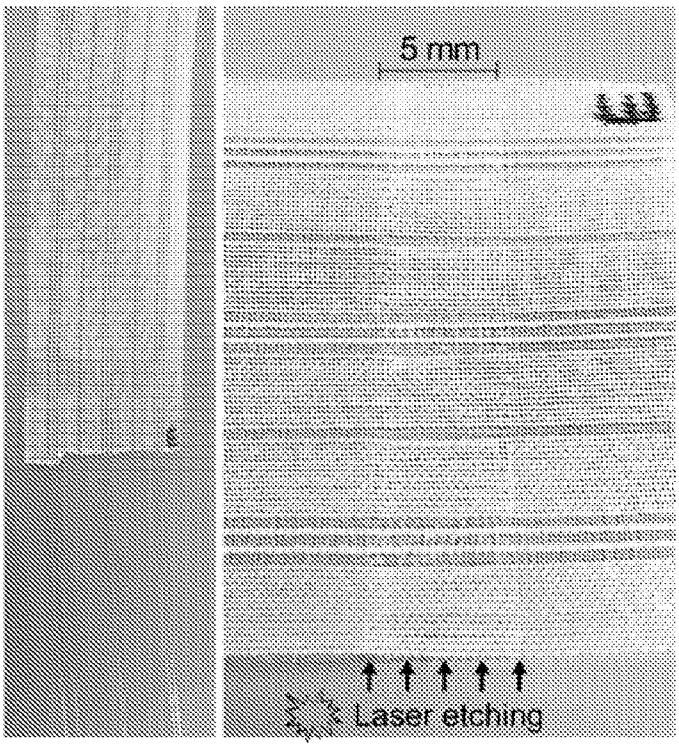


FIG. 17A

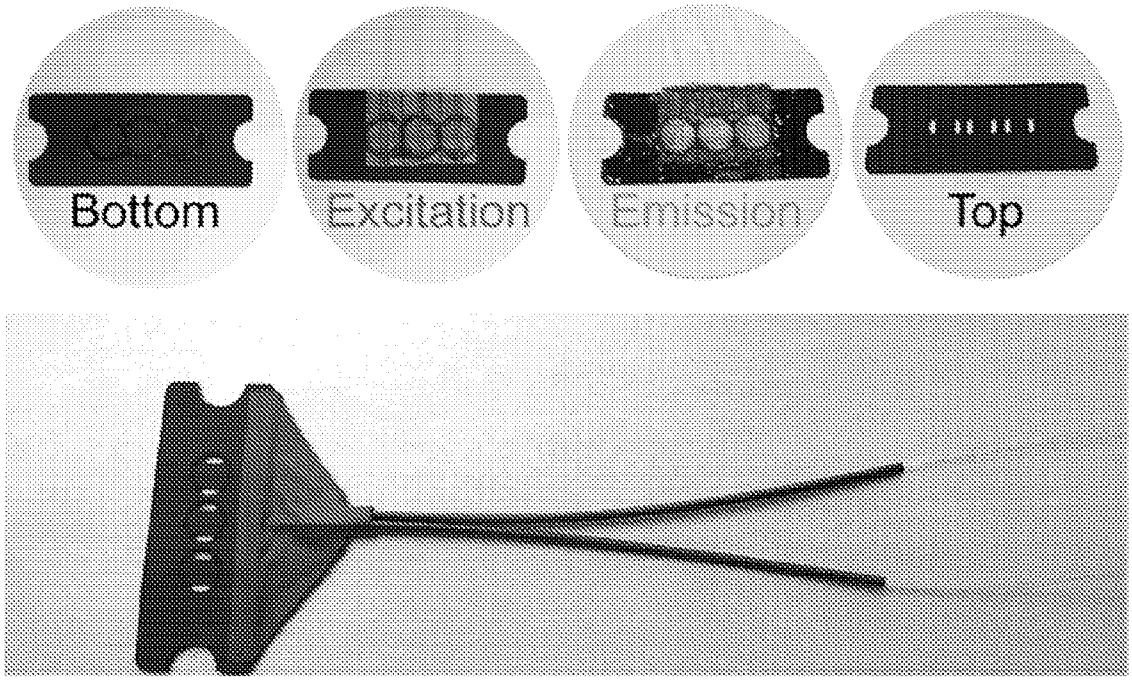


FIG. 17B

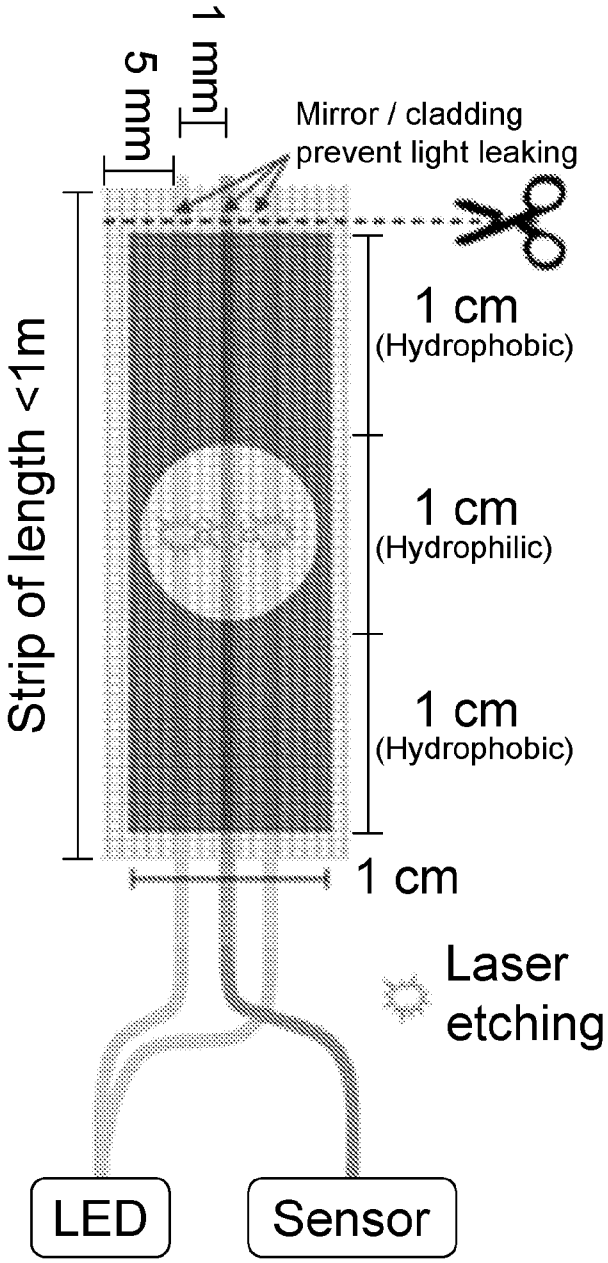

5 mm
1 mm
Mirror / cladding
prevent light leaking
Strip of length <1m
1 cm
(Hydrophobic)
1 cm
(Hydrophilic)
1 cm
(Hydrophobic)
1 cm
Laser
etching
LED
Sensor

FIG. 17C

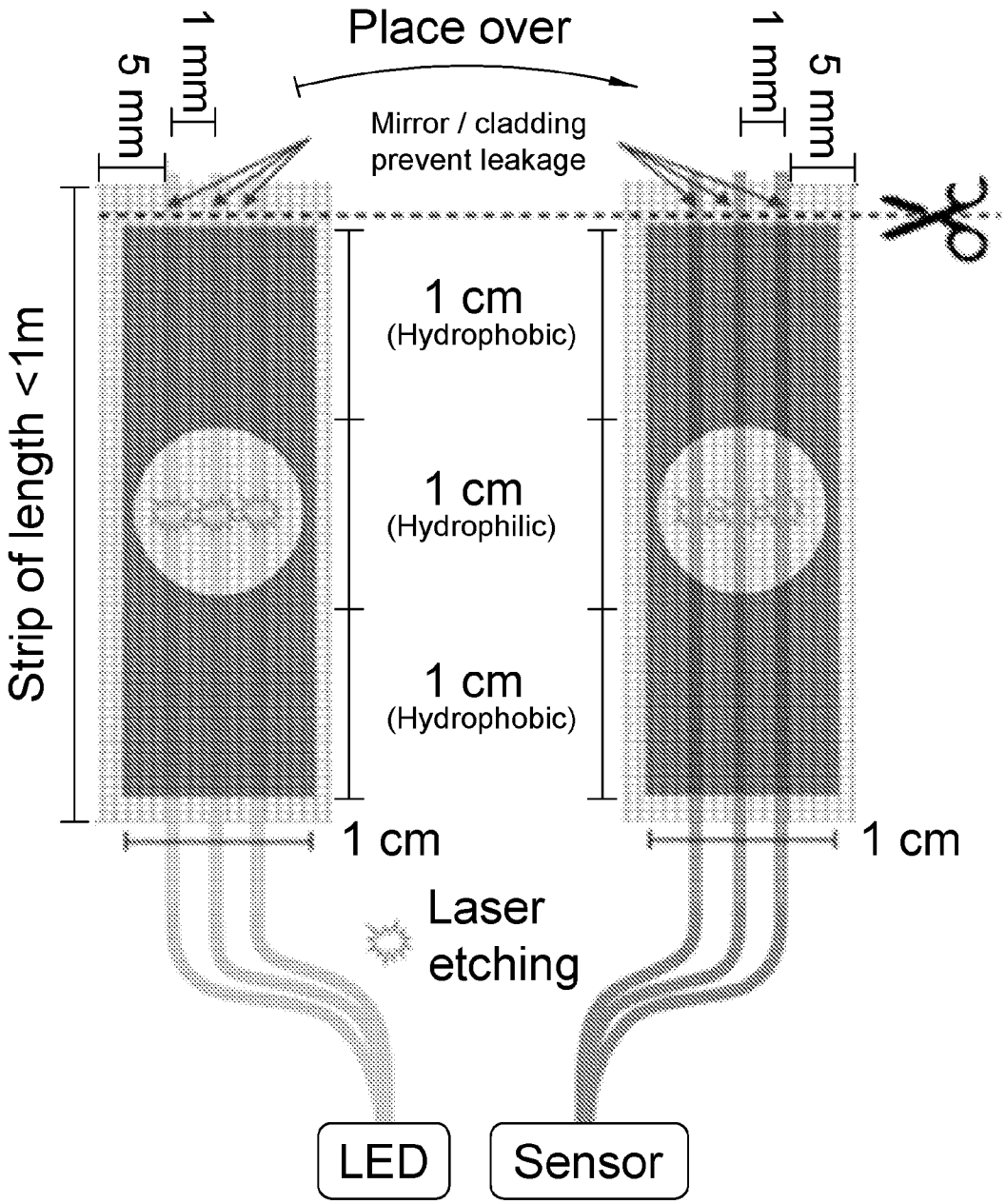

Place over
Mirror / cladding
prevent leakage
1 mm
5 mm
1 mm
5 mm
Strip of length <1m
1 cm
(Hydrophobic)
1 cm
(Hydrophilic)
1 cm
(Hydrophobic)
1 cm
1 cm
Laser
etching
LED
Sensor

FIG. 17D

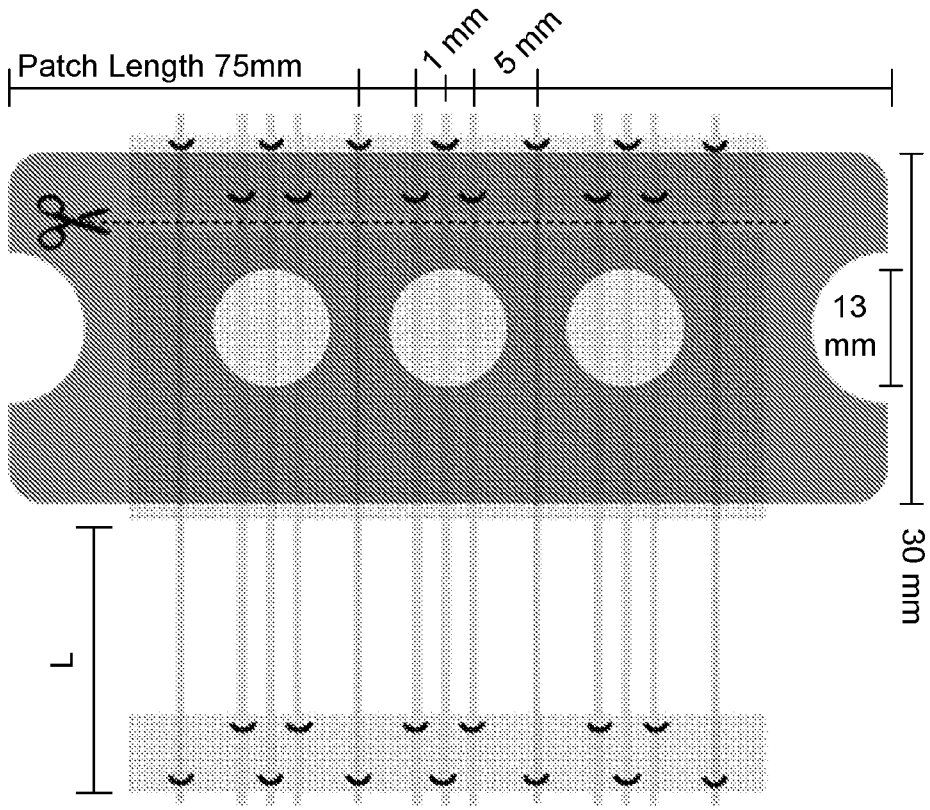


FIG. 17E

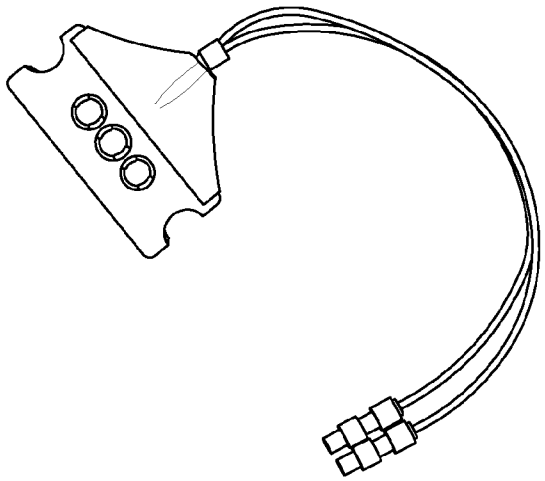


FIG. 17F

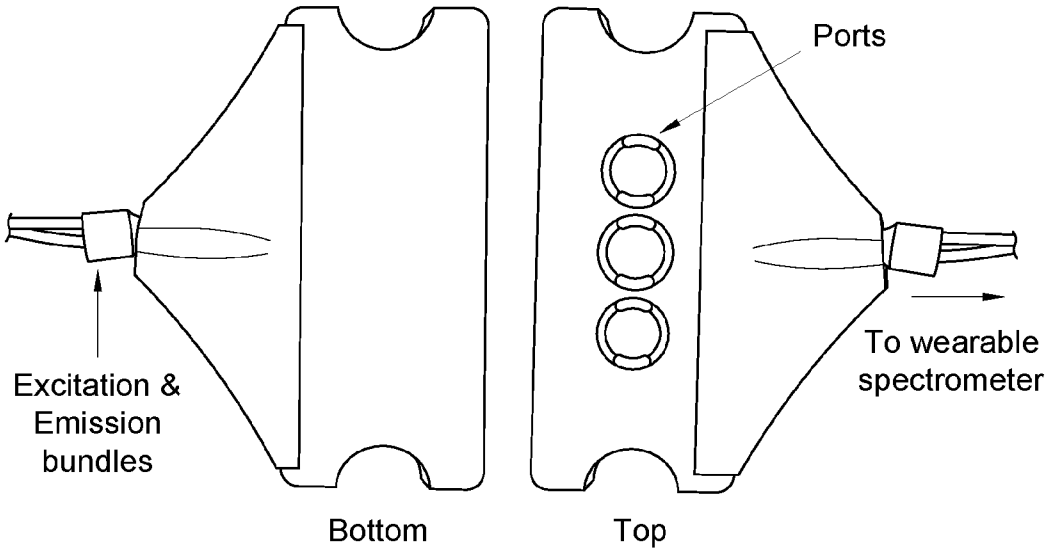


FIG. 17G

FIG. 18A

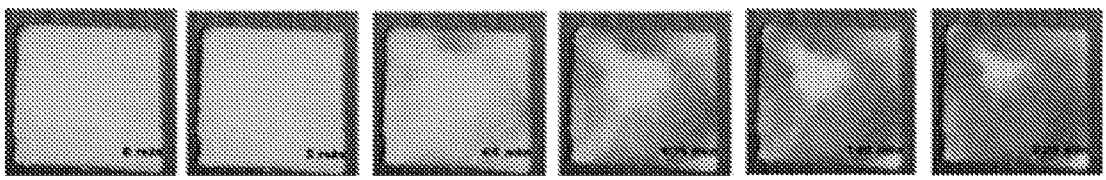


FIG. 18B

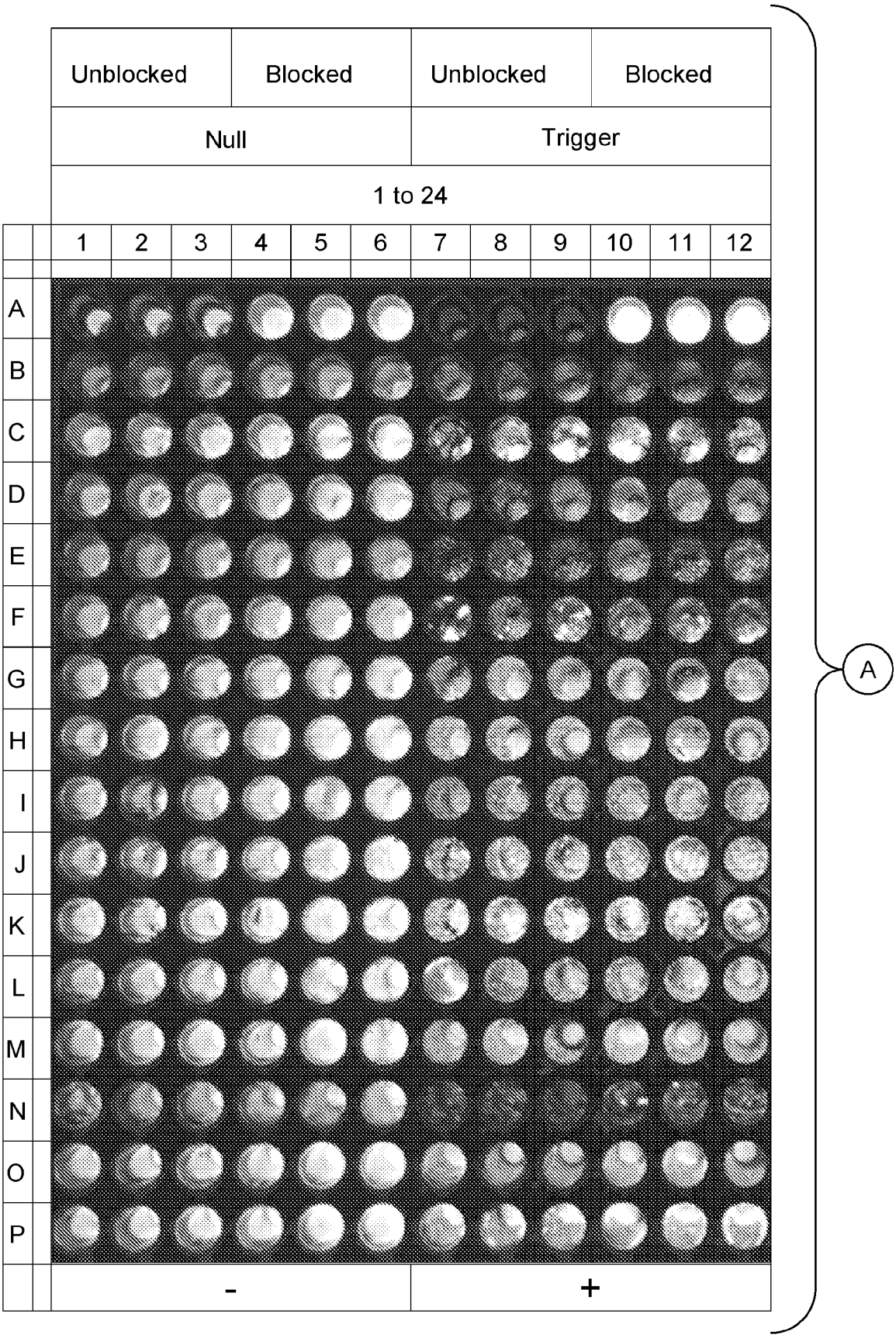

Unblocked
Blocked
Unblocked
Blocked
Null
Trigger
1 to 24
1 2 3 4 5 6 7 8 9 10 11 12
A B C D E F G H I J K L M N O P
A
- +

FIG. 19A

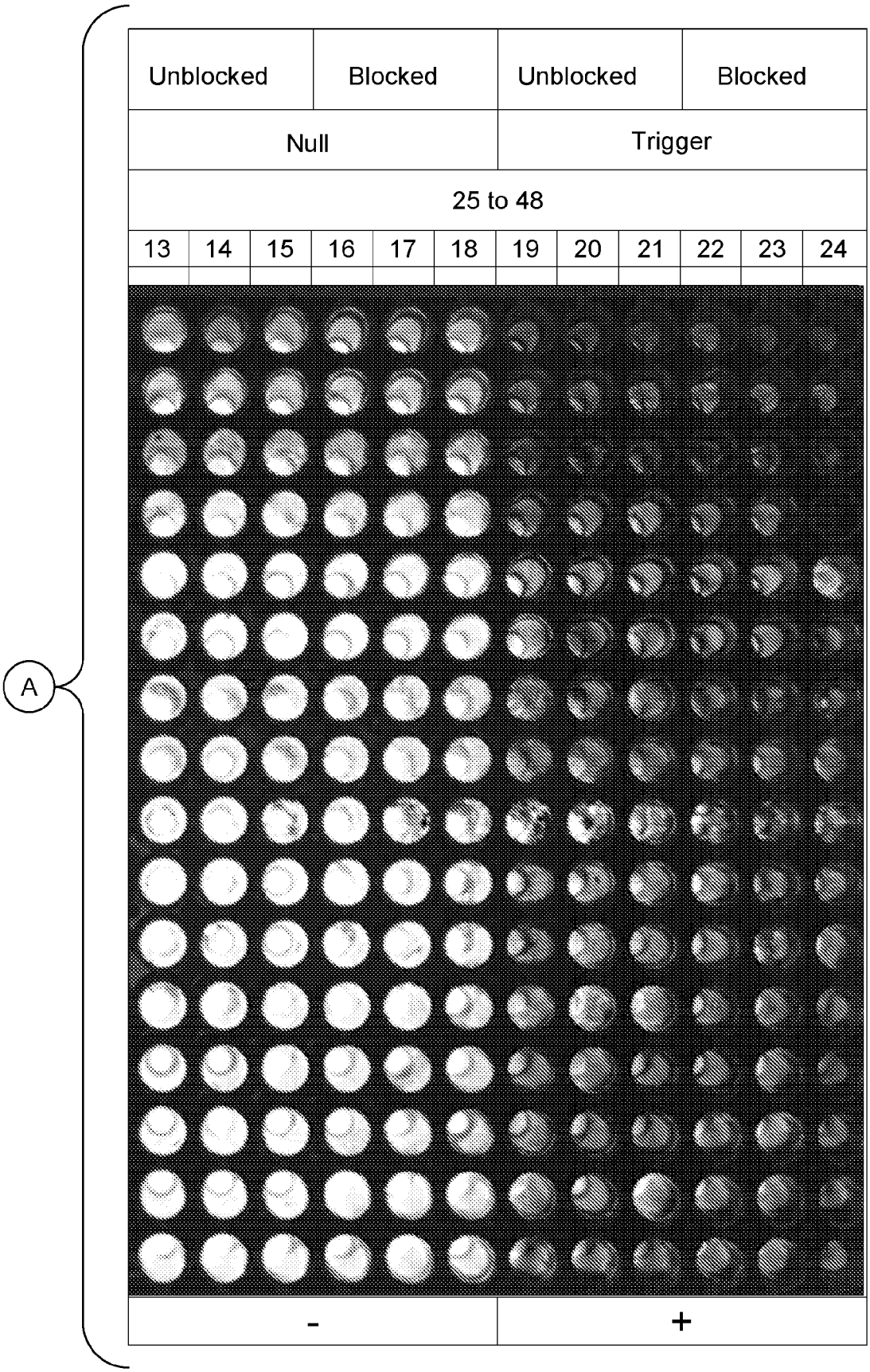


FIG. 19A (CONTINUATION)

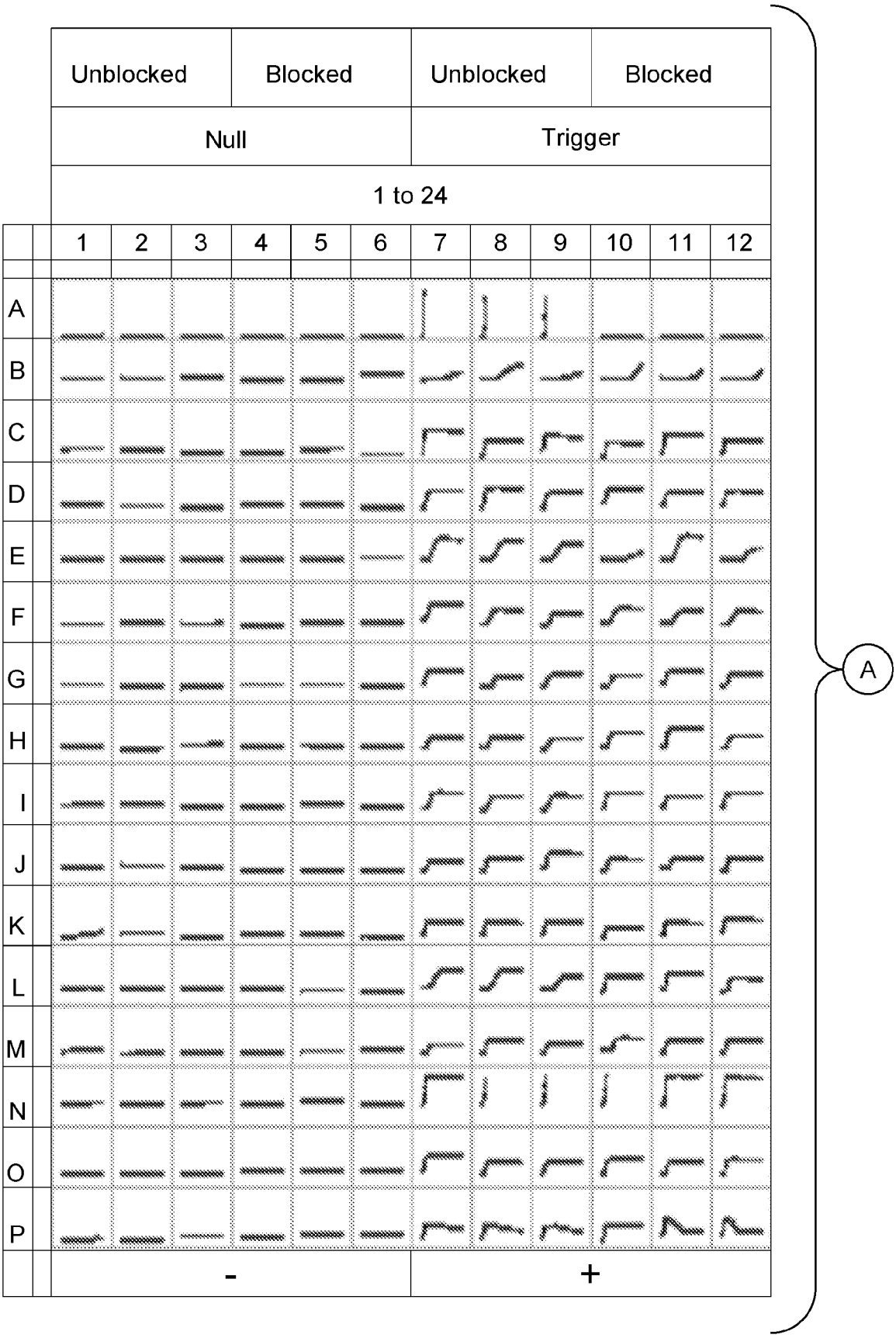

Unblocked
Blocked
Unblocked
Blocked
Null
Trigger
1 to 24
1
2
3
4
5
6
7
8
9
10
11
12
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
-
+
A

FIG. 19B

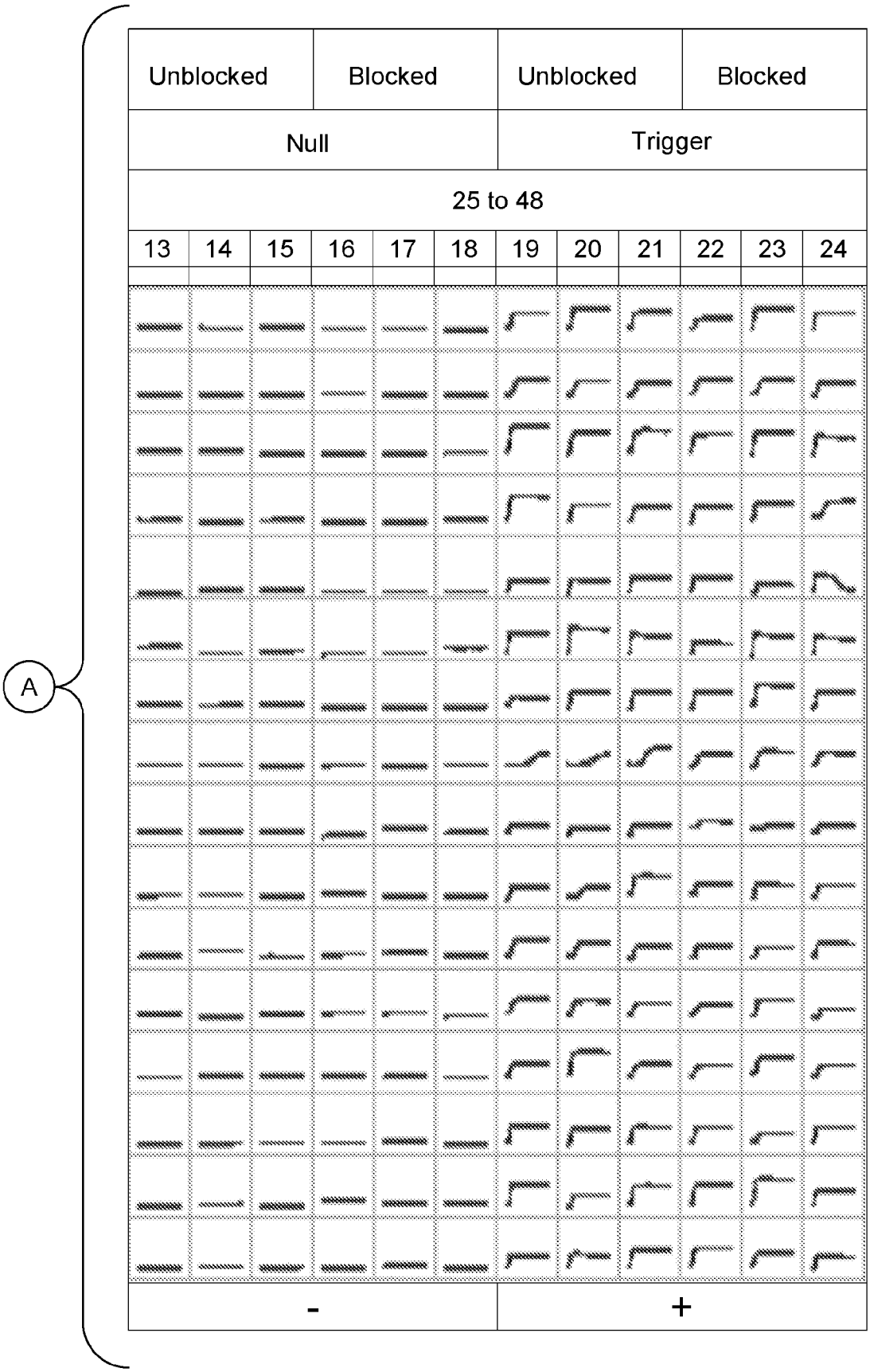


FIG. 19B (CONTINUATION)

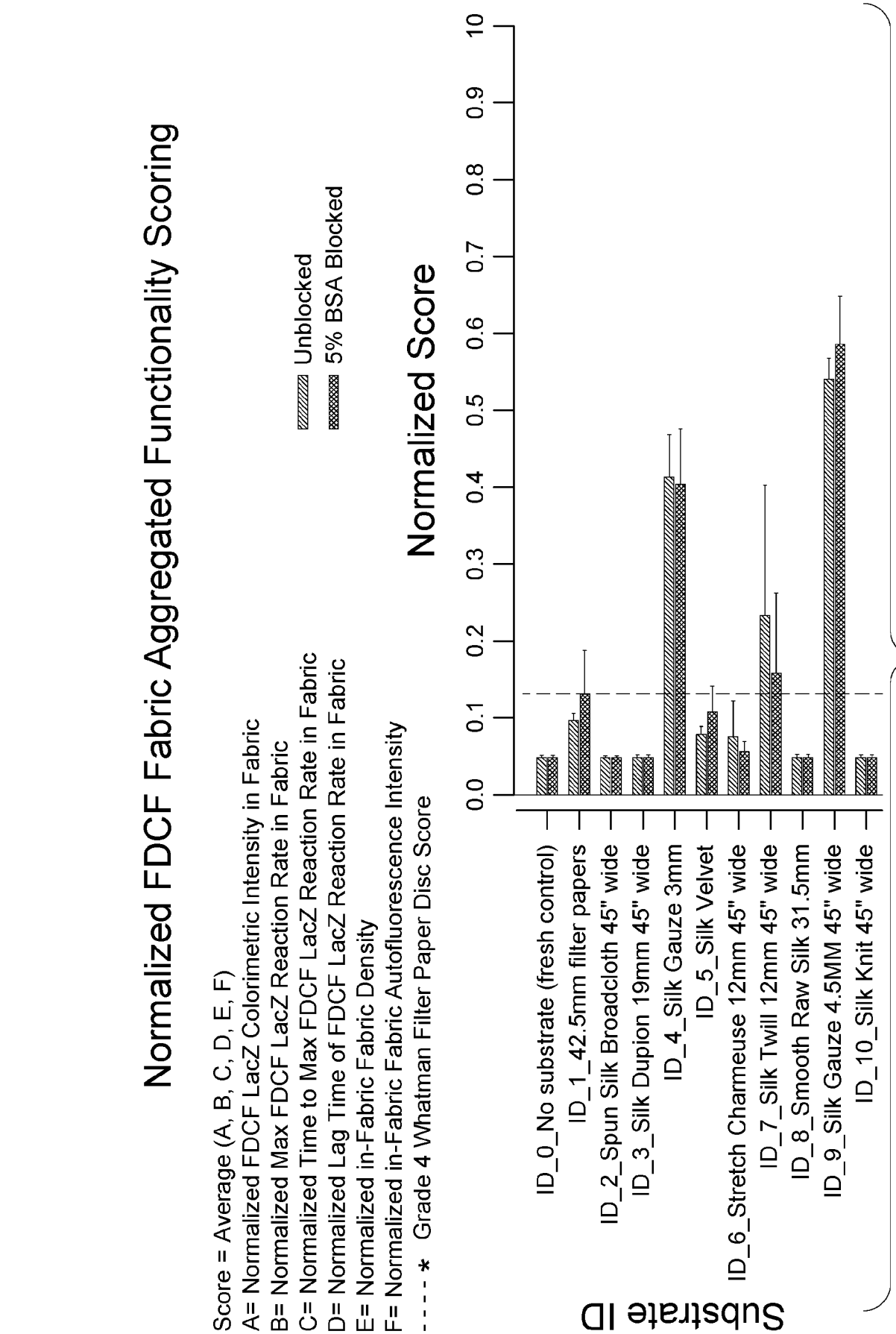

Normalized FDCF Fabric Aggregated Functionality Scoring
Score = Average (A, B, C, D, E, F)
A= Normalized FDCF LacZ Colorimetric Intensity in Fabric
B= Normalized Max FDCF LacZ Reaction Rate in Fabric
C= Normalized Time to Max FDCF LacZ Reaction Rate in Fabric
D= Normalized Lag Time of FDCF LacZ Reaction Rate in Fabric
E= Normalized in-Fabric Fabric Density
F= Normalized in-Fabric Fabric Autofluorescence Intensity
- - - - * Grade 4 Whatman Filter Paper Disc Score
Unblocked
5% BSA Blocked
Normalized Score
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
Substrate ID
ID_0_No substrate (fresh control)
ID_1_42.5mm filter papers
ID_2_Spun Silk Broadcloth 45" wide
ID_3_Silk Dupion 19mm 45" wide
ID_4_Silk Gauze 3mm
ID_5_Silk Velvet
ID_6_Stretch Charmeuse 12mm 45" wide
ID_7_Silk Twill 12mm 45" wide
ID_8_Smooth Raw Silk 31.5mm
ID_9_Silk Gauze 4.5MM 45" wide
ID_10_Silk Knit 45" wide
A

FIG. 20

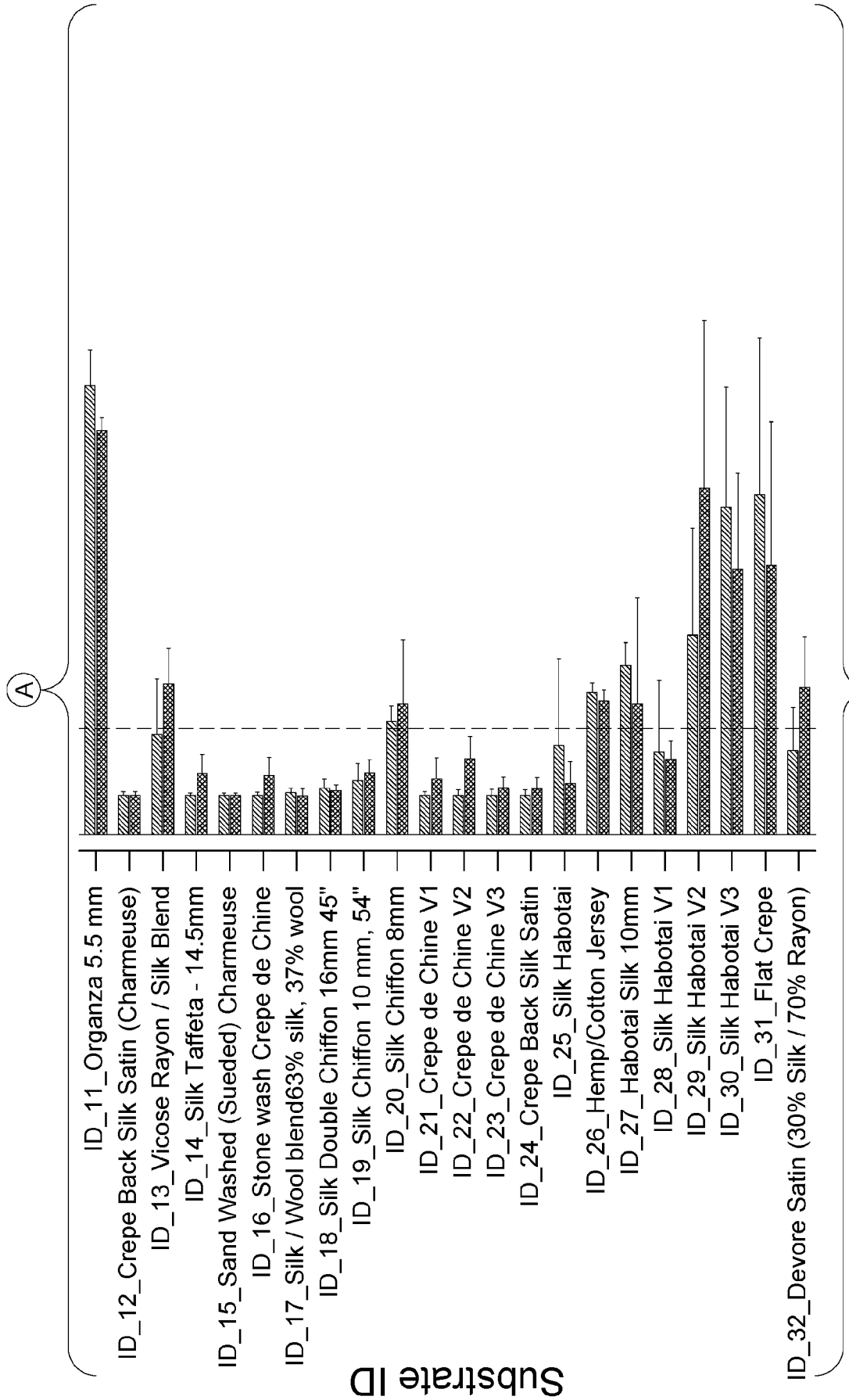


FIG. 20 (CONTINUATION)

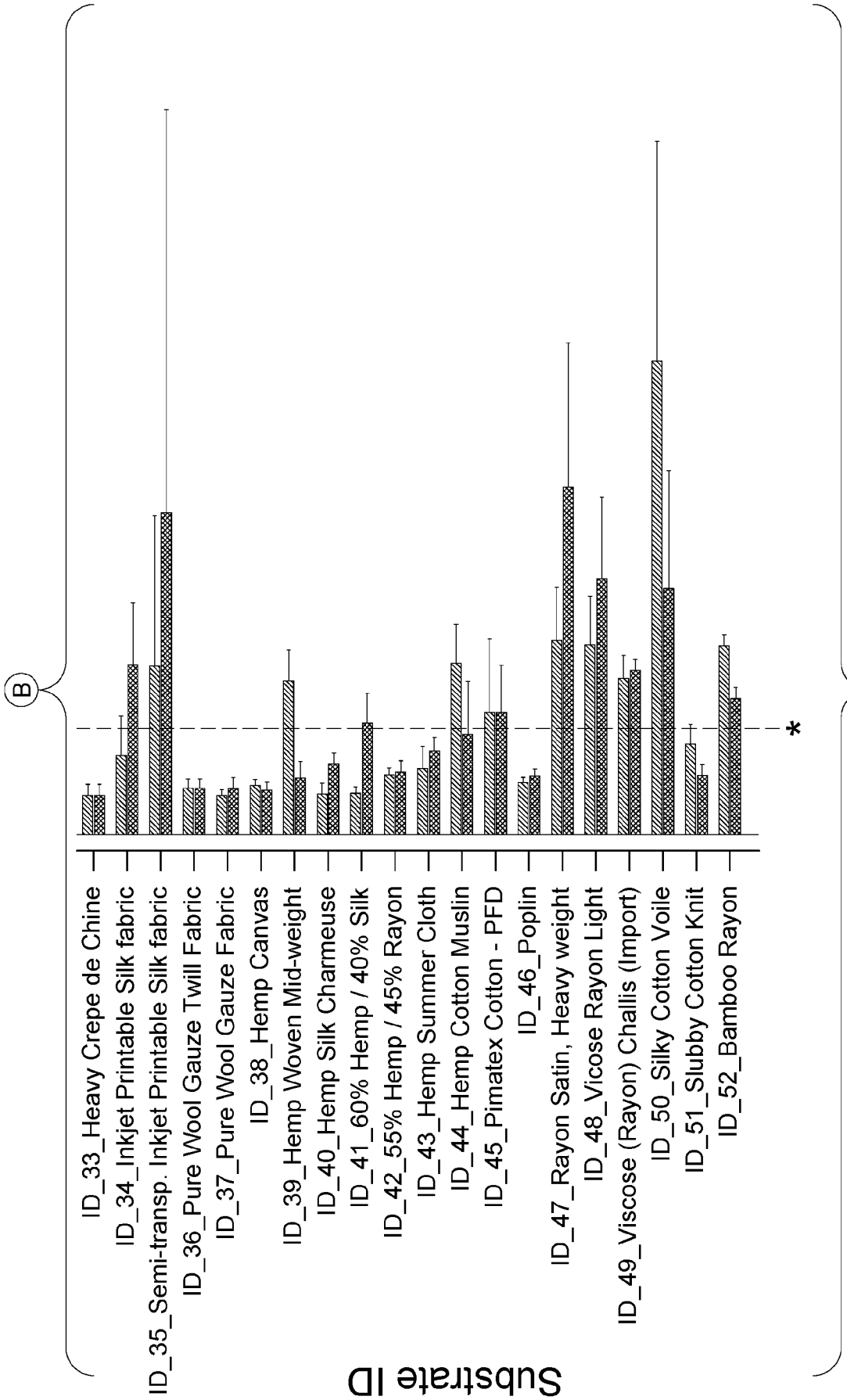


FIG. 20 (CONTINUATION)

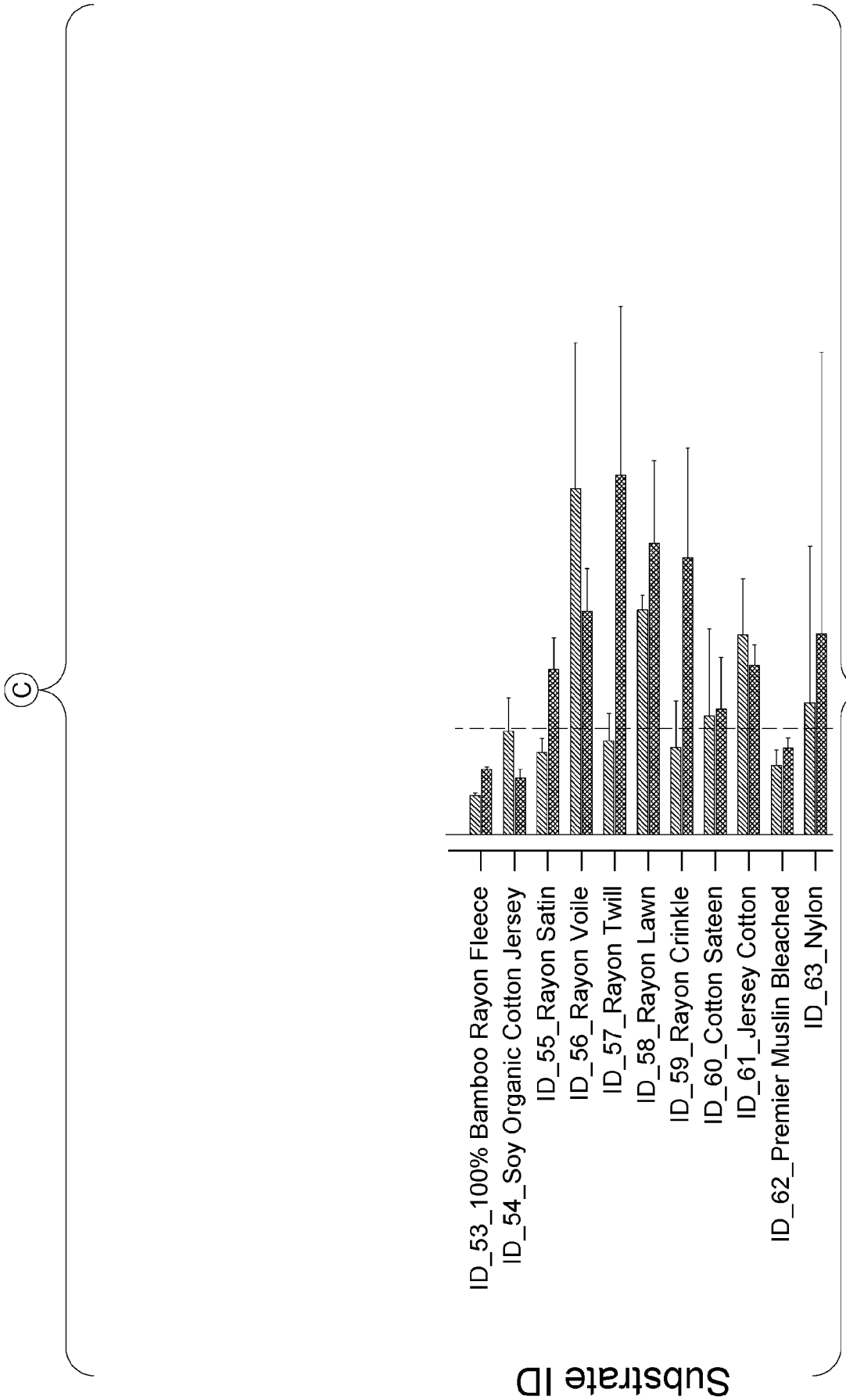


FIG. 20 (CONTINUATION)

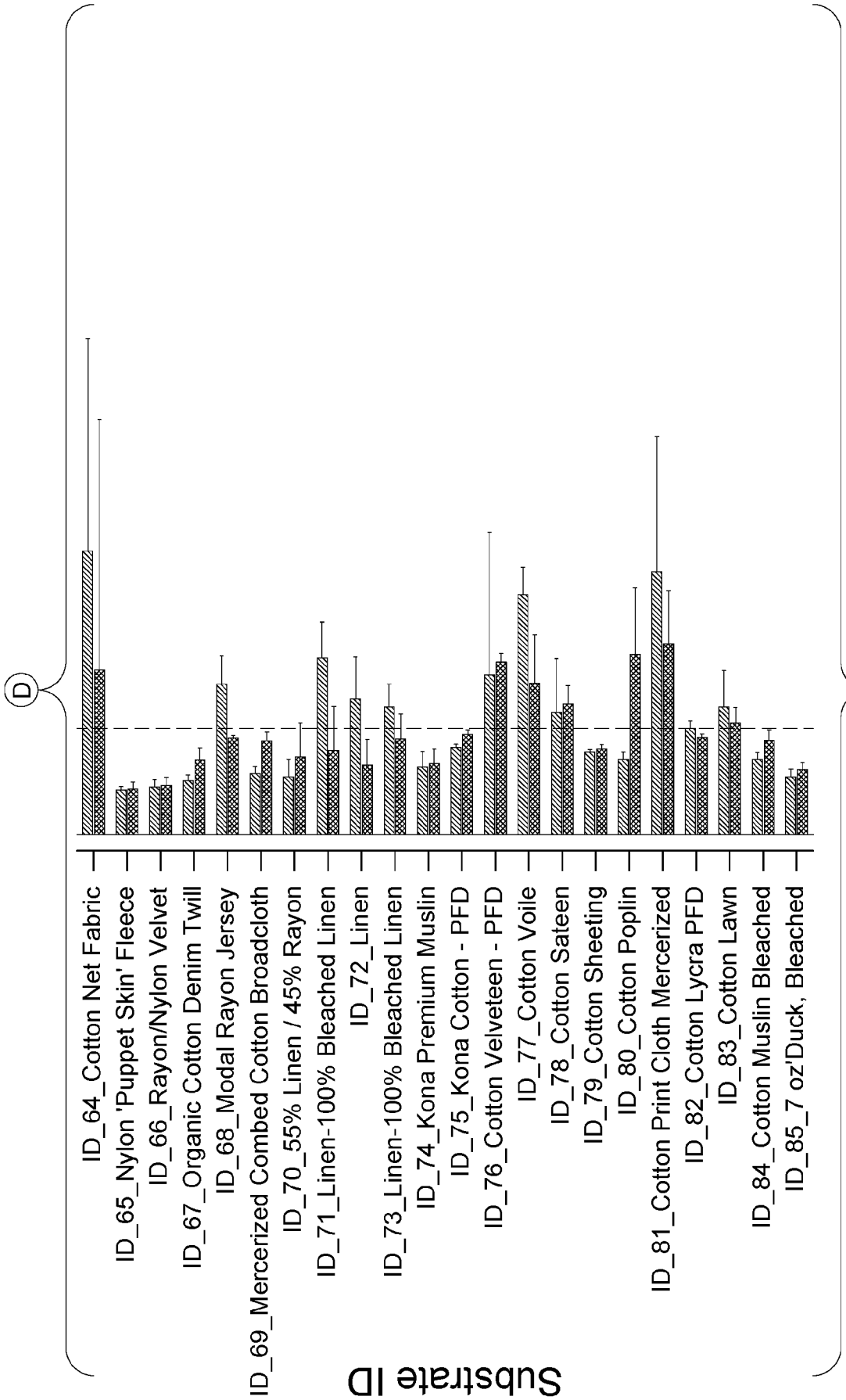


FIG. 20 (CONTINUATION)

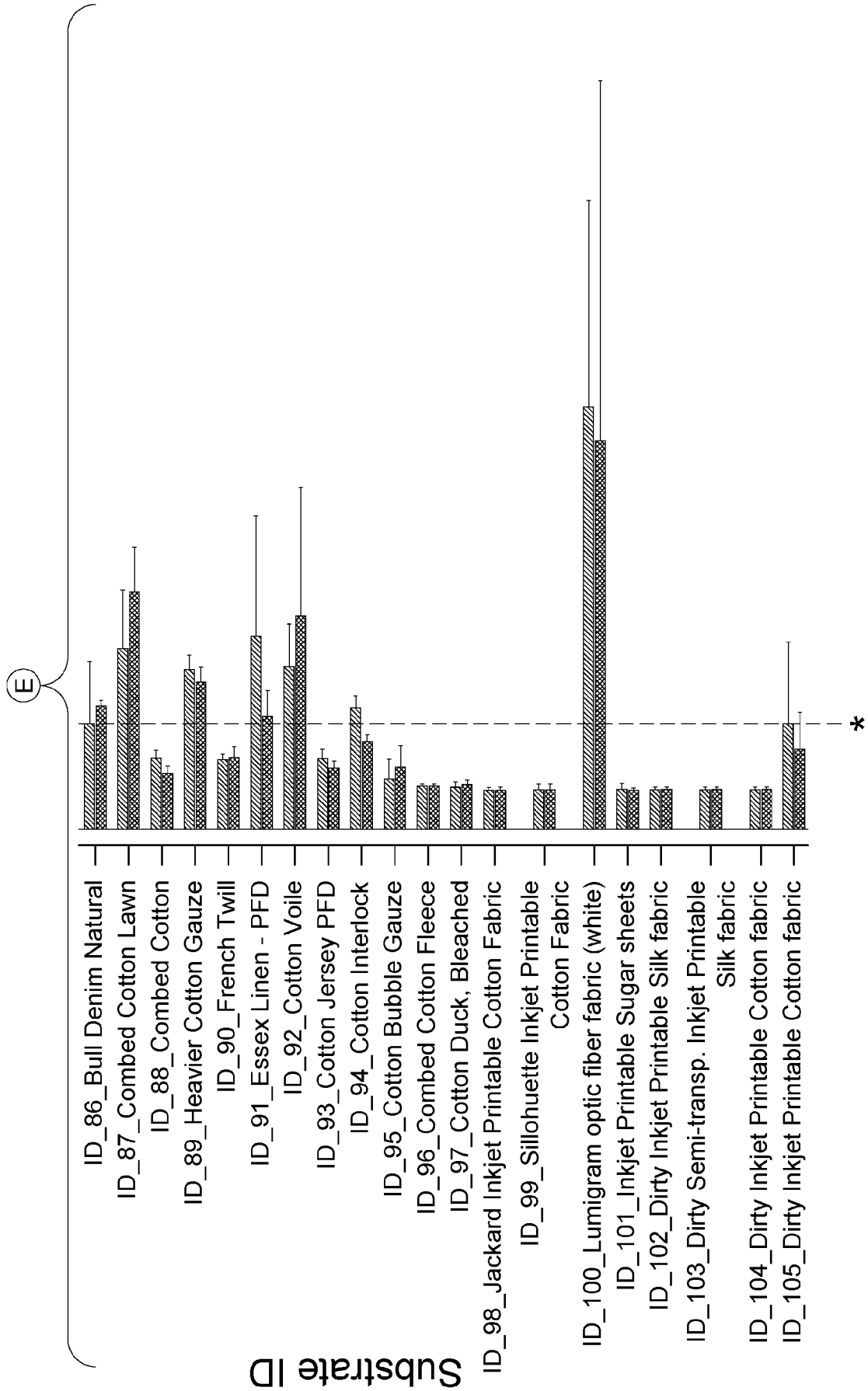


FIG. 20 (CONTINUATION)

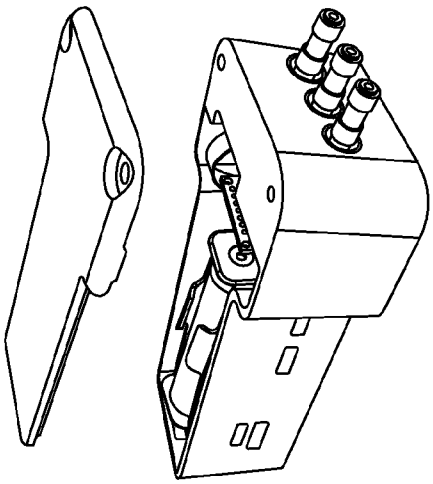


FIG. 21B

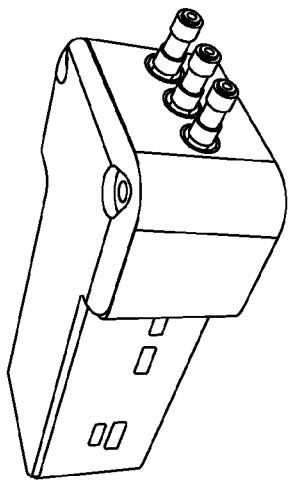


FIG. 21C

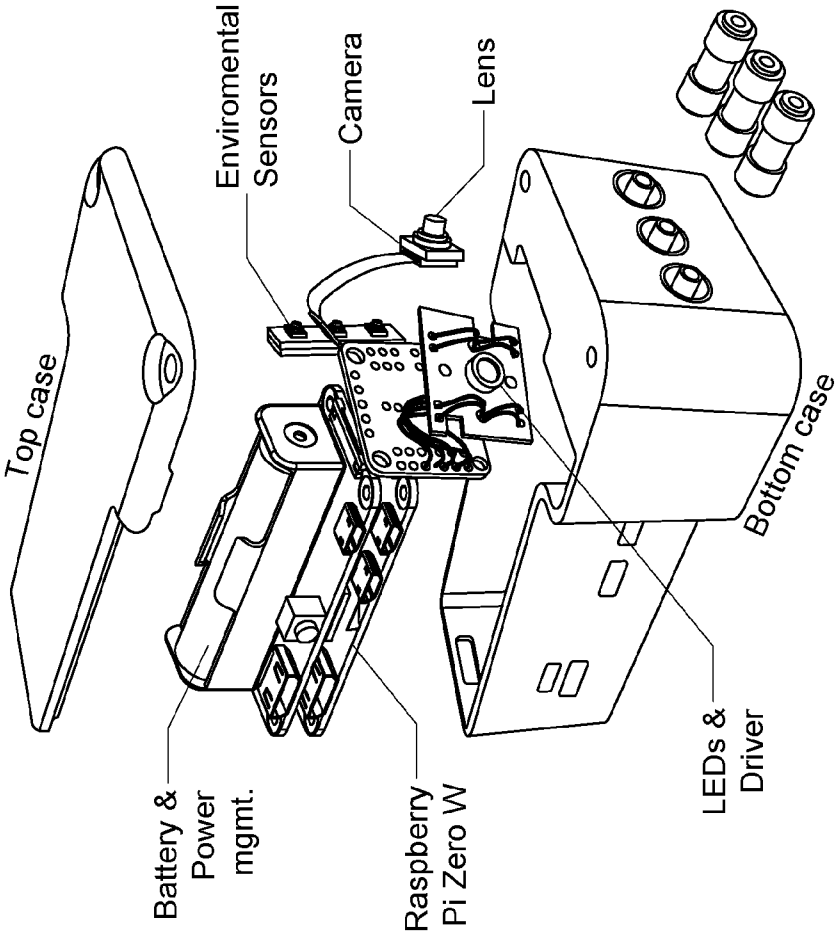


FIG. 21A

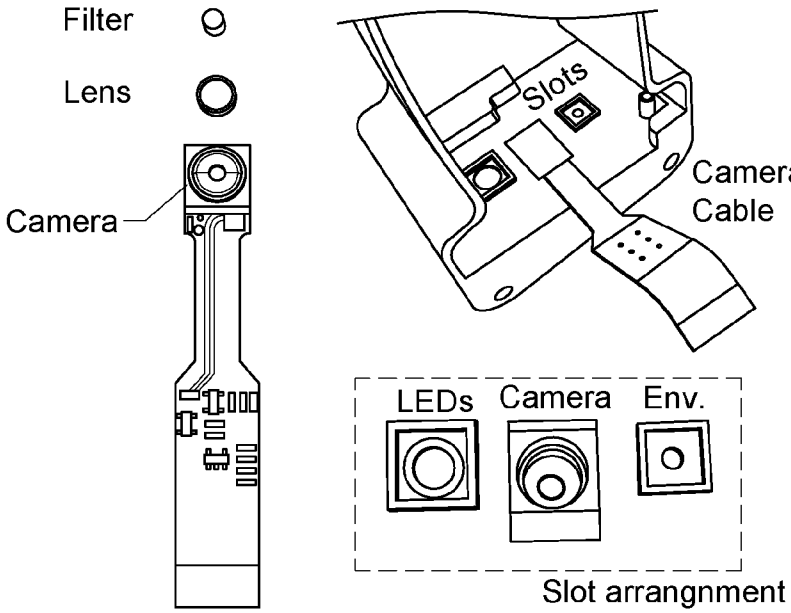


FIG. 21D

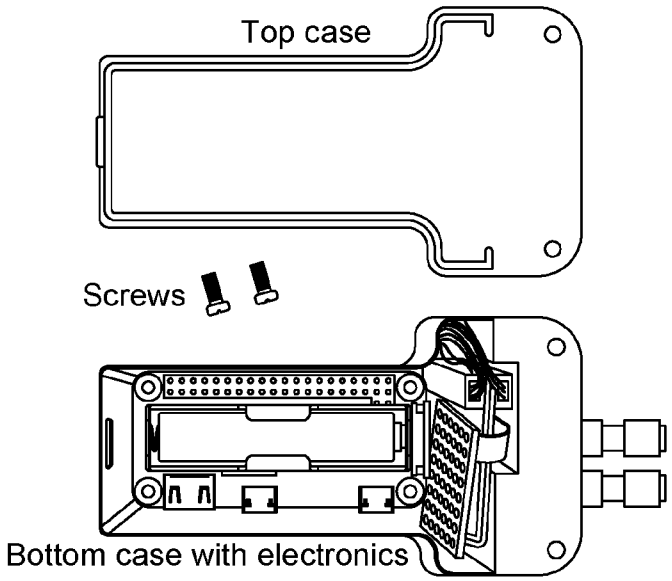


FIG. 21E

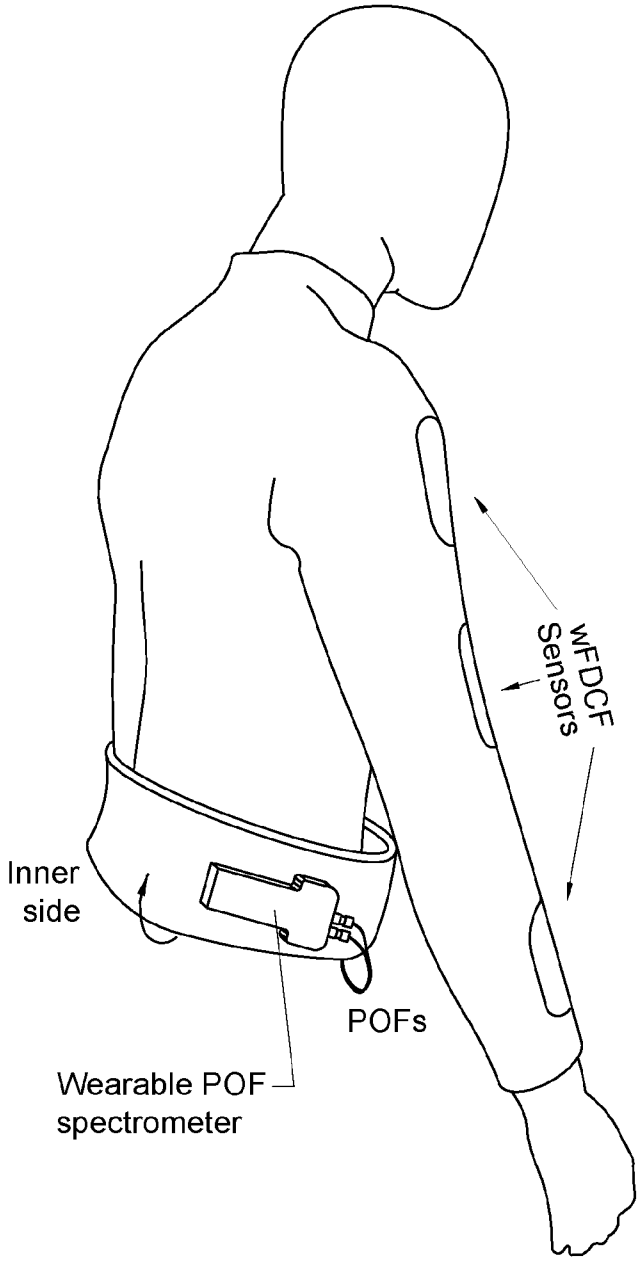


FIG. 21F

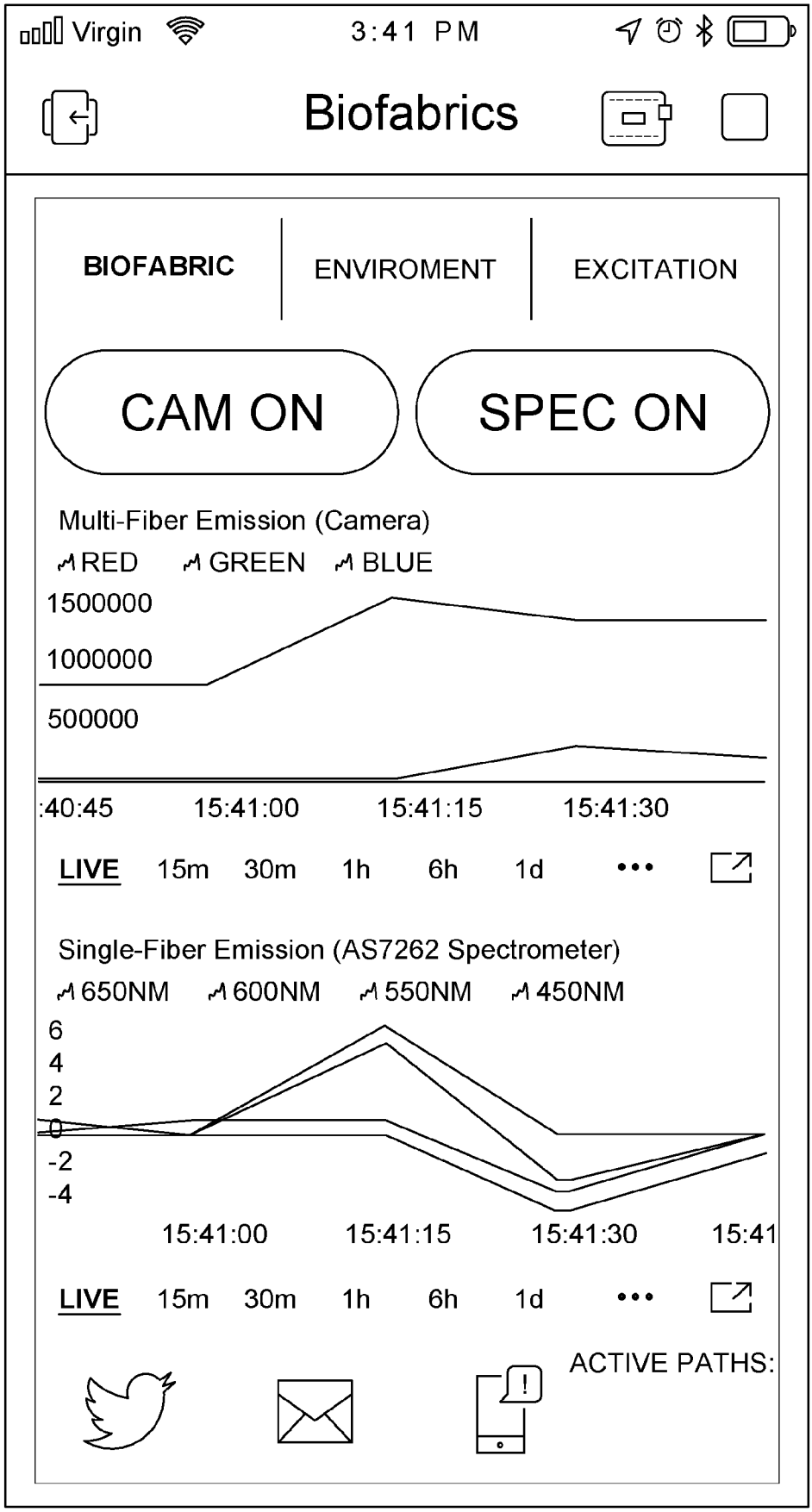

Virgin
3:41 PM
Biofabrics
BIOFABRIC
ENVIROMENT
EXCITATION
CAM ON
SPEC ON
Multi-Fiber Emission (Camera)
RED
GREEN
BLUE
1500000
1000000
500000
:40:45
15:41:00
15:41:15
15:41:30
LIVE
15m
30m
1h
6h
1d
Single-Fiber Emission (AS7262 Spectrometer)
650NM
600NM
550NM
450NM
6
4
2
0
-2
-4
15:41:00
15:41:15
15:41:30
15:41
LIVE
15m
30m
1h
6h
1d
ACTIVE PATHS:

FIG. 22A

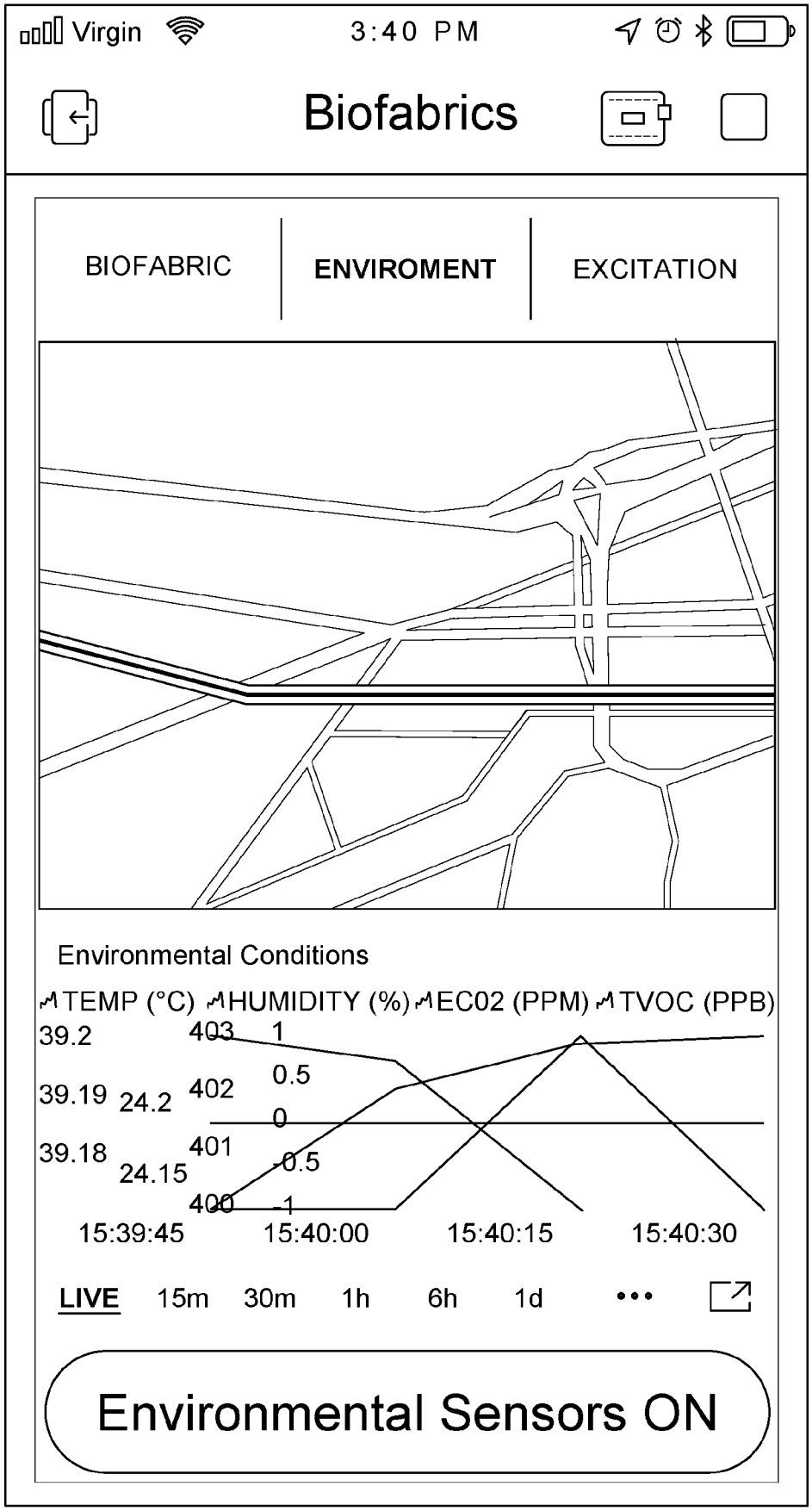

Virgin
3:40 PM
Biofabrics
BIOFABRIC
ENVIROMENT
EXCITATION
Environmental Conditions
TEMP (°C)
HUMIDITY (%)
EC02 (PPM)
TVOC (PPB)
39.2
39.19
24.2
39.18
24.15
403
402
401
400
1
0.5
0
-0.5
-1
15:39:45
15:40:00
15:40:15
15:40:30
LIVE
15m
30m
1h
6h
1d
Environmental Sensors ON

FIG. 22B

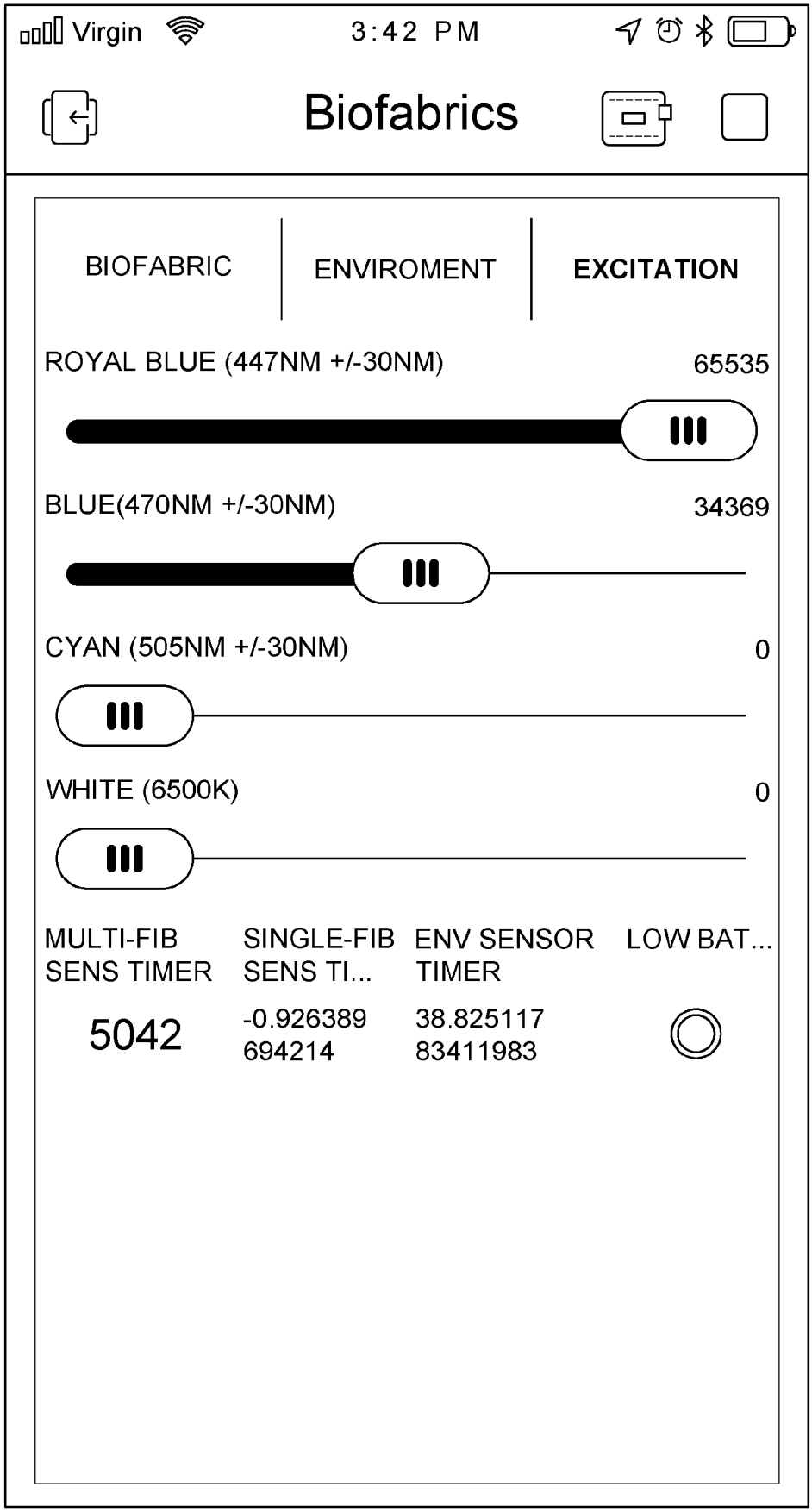

Virgin
3:42 PM
Biofabrics
BIOFABRIC
ENVIROMENT
EXCITATION
ROYAL BLUE (447NM +/-30NM)
65535
BLUE(470NM +/-30NM)
34369
CYAN (505NM +/-30NM)
0
WHITE (6500K)
0
MULTI-FIB SENS TIMER
SINGLE-FIB SENS TI...
ENV SENSOR TIMER
LOW BAT...
5042
-0.926389694214
38.82511783411983

FIG. 22C

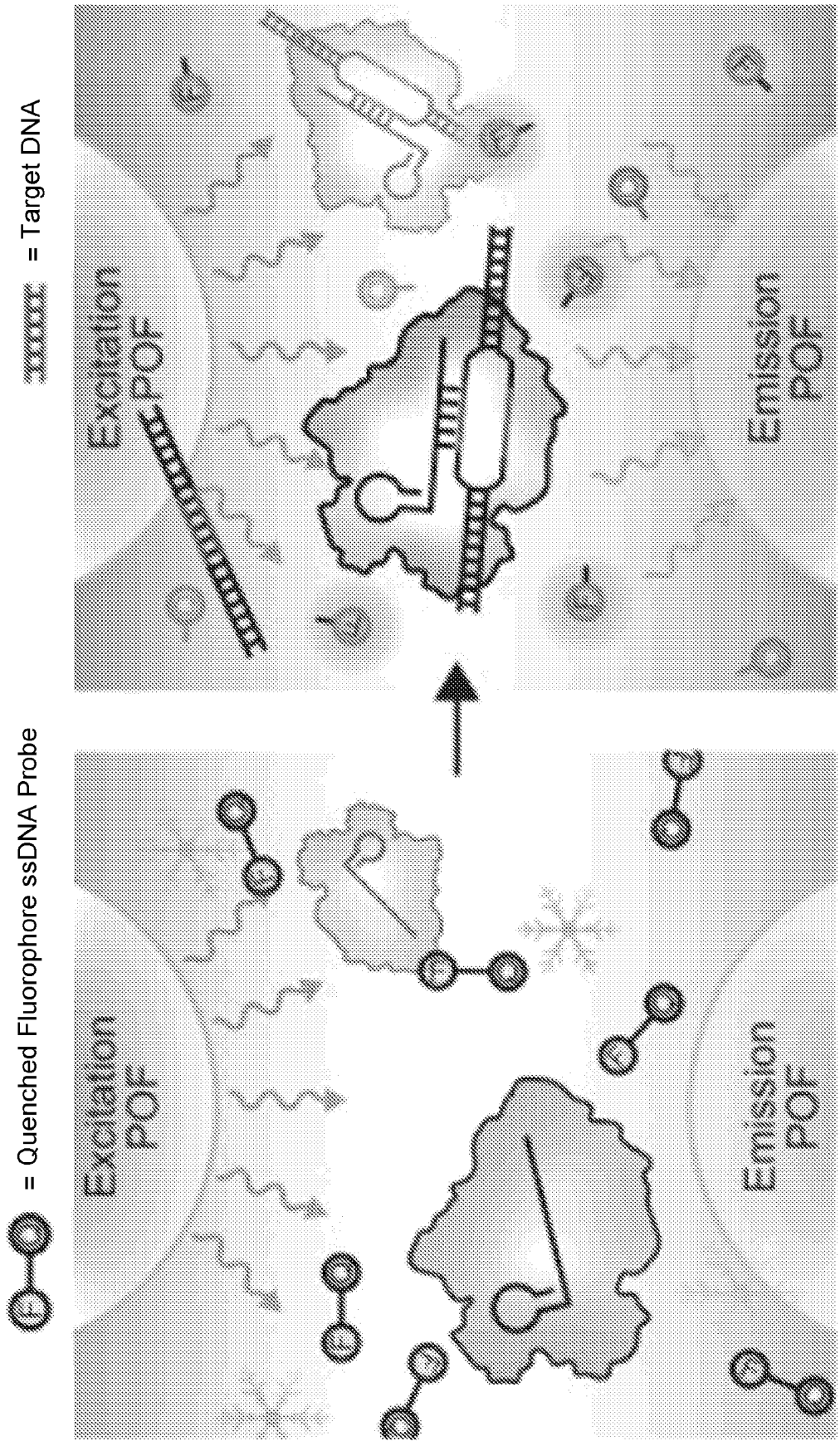

= Quenched Fluorophore ssDNA Probe
= Target DNA
Excitation POF
Emission POF
Excitation POF
Emission POF

FIG. 23A

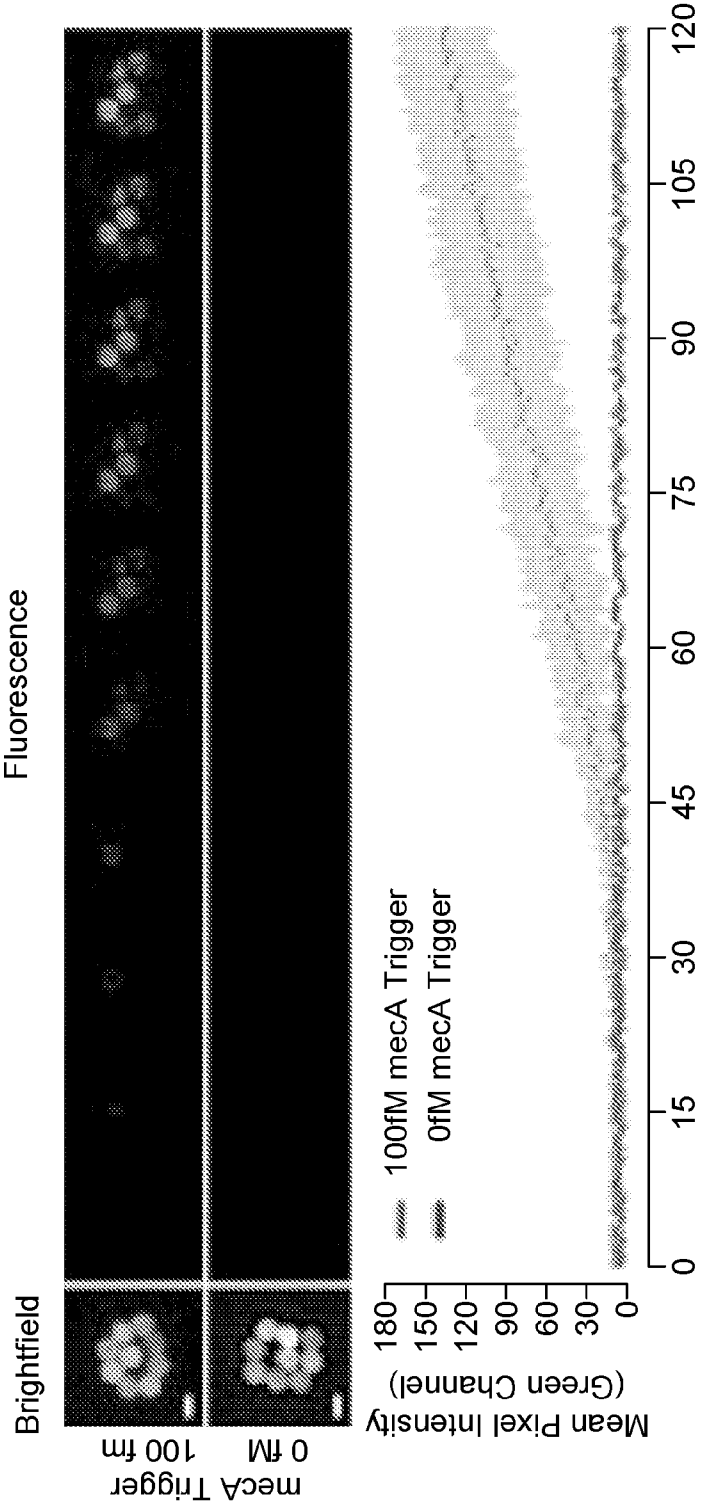

Brightfield
Fluorescence
mecA Trigger
100 fm
0 fM
100fM mecA Trigger
0fM mecA Trigger
Mean Pixel Intensity
(Green Channel)
180
150
120
90
60
30
0
0
15
30
45
60
75
90
105
120
Time (min)

FIG. 23B

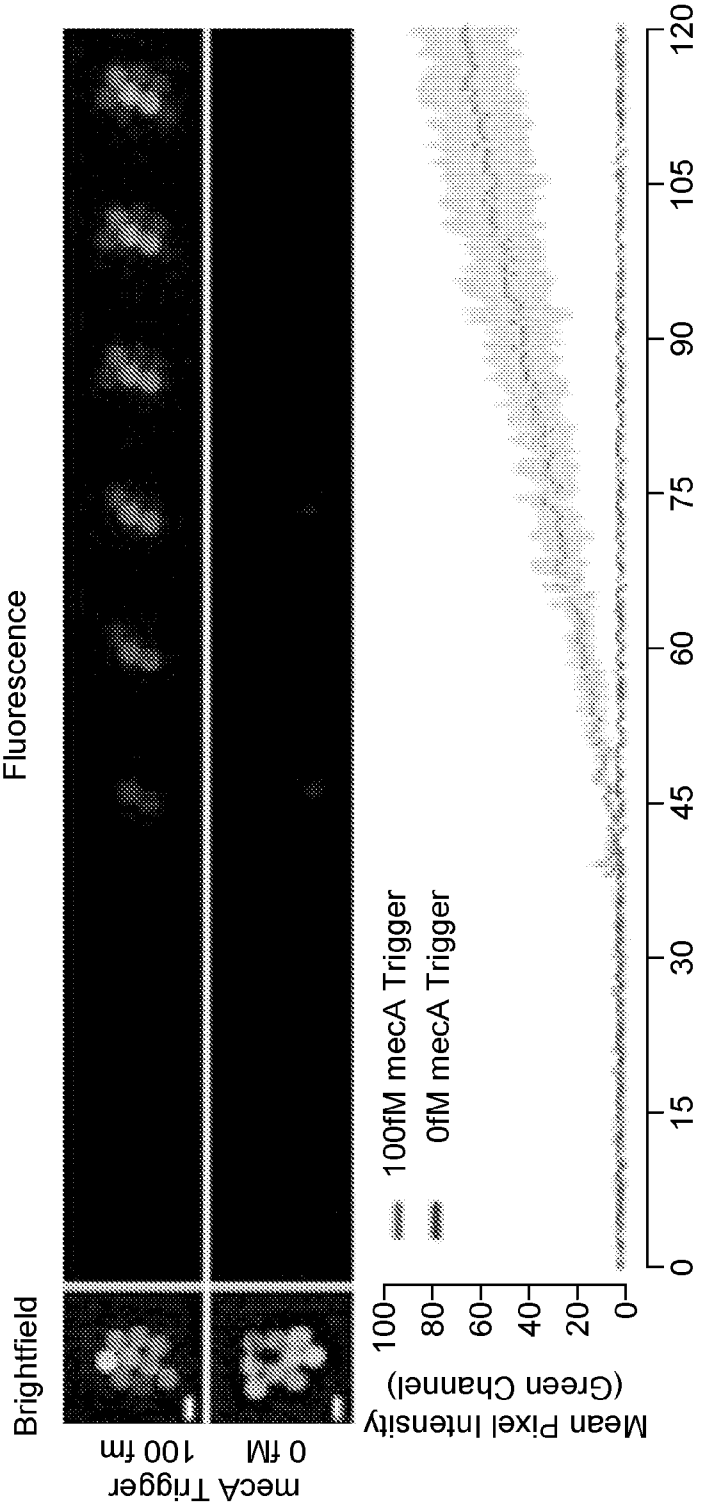

Brightfield
Fluorescence
mecA Trigger
0 fM
100 fm
Mean Pixel Intensity
(Green Channel)
100fM mecA Trigger
0fM mecA Trigger
0
20
40
60
80
100
0
15
30
45
60
75
90
105
120
Time (min)

FIG. 23C

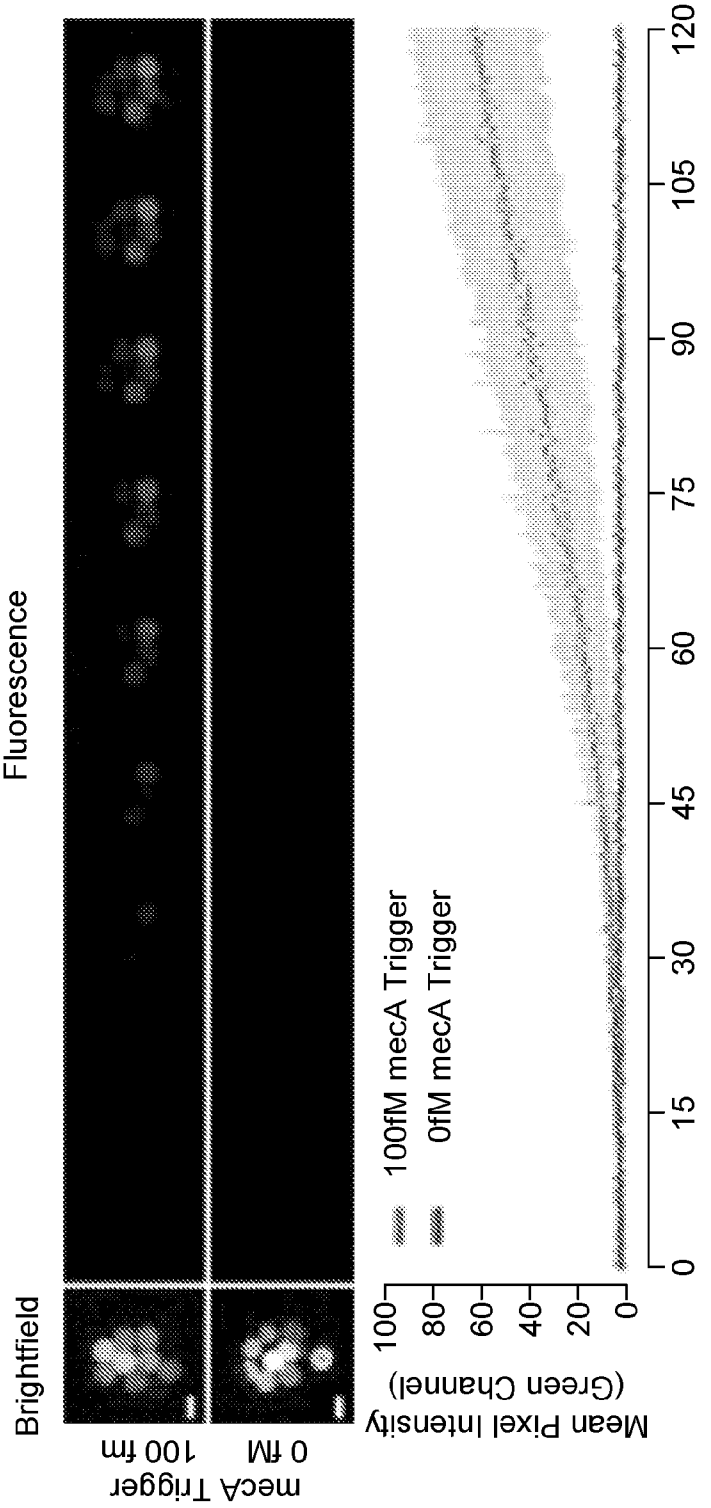

Brightfield
Fluorescence
mecA Trigger
0 fM
100 fm
100fM mecA Trigger
0fM mecA Trigger
Mean Pixel Intensity
(Green Channel)
100
80
60
40
20
0
0
15
30
45
60
75
90
105
120
Time (min)

FIG. 23D

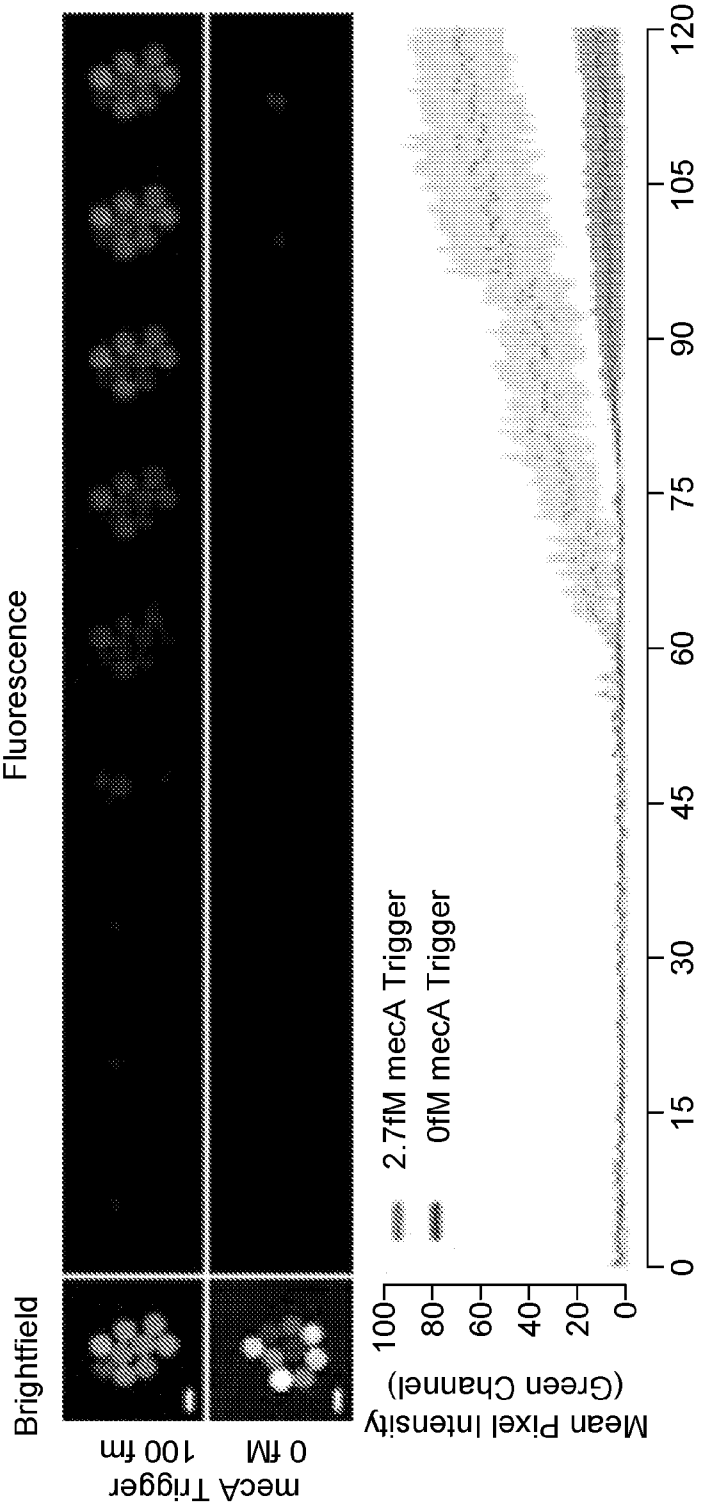

Brightfield
Fluorescence
mecA Trigger
0 fM
100 fm
Mean Pixel Intensity
(Green Channel)
100
80
60
40
20
0
2.7fM mecA Trigger
0fM mecA Trigger
0
15
30
45
60
75
90
105
120
Time (min)

FIG. 23E

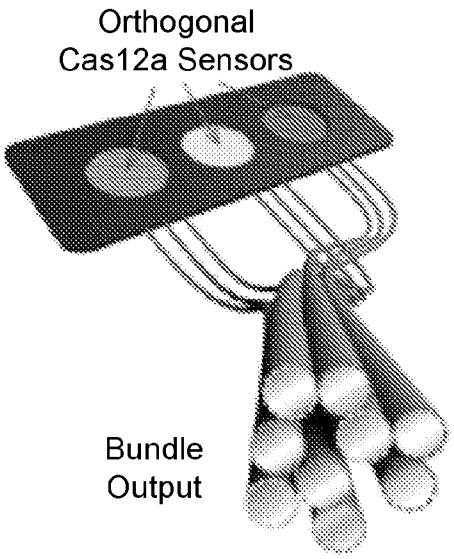


FIG. 23F

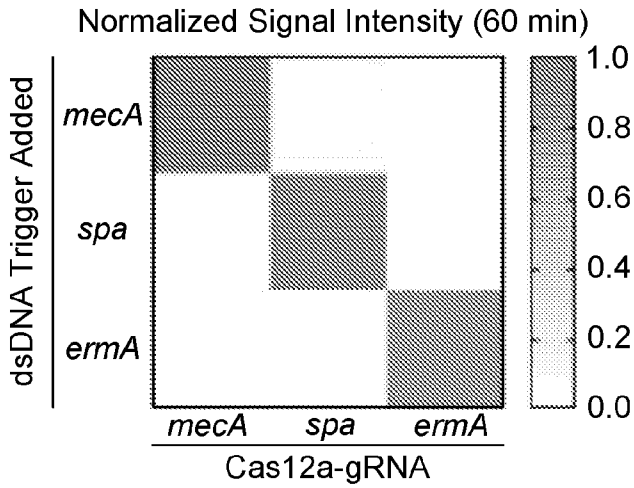


FIG. 23G

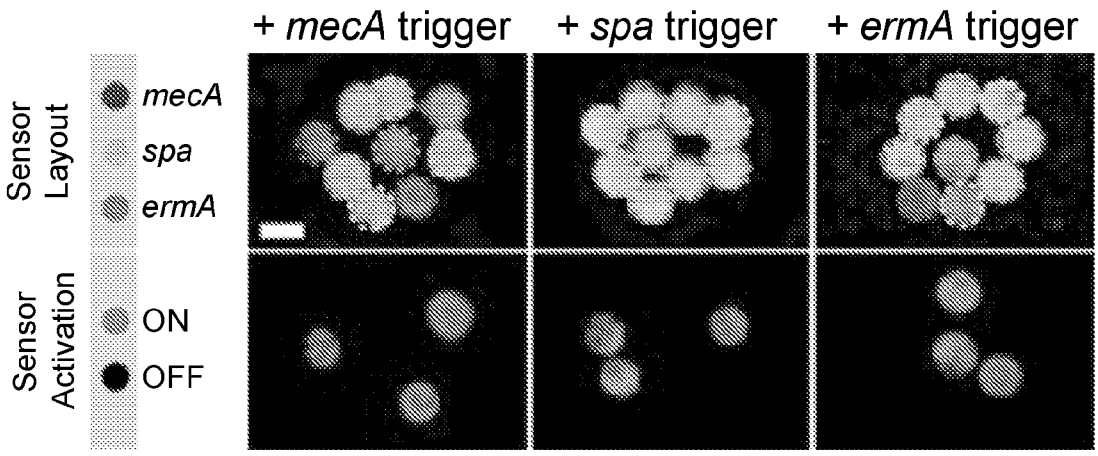


FIG. 23H

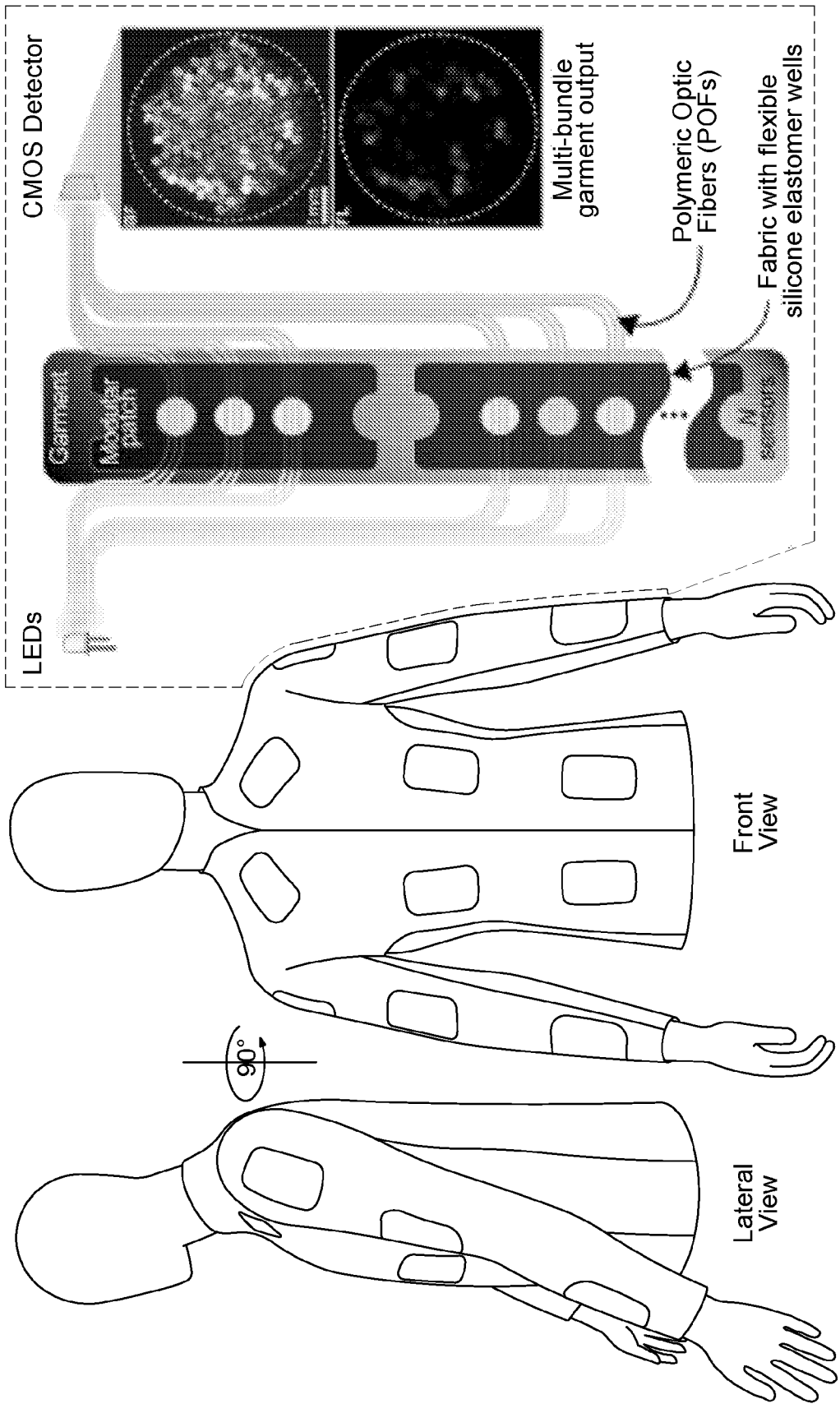

CMOS Detector
Multi-bundle garment output
Polymeric Optic Fibers (POFs)
Fabric with flexible silicone elastomer wells
LEDs
Front View
Lateral View
90°

FIG. 23I

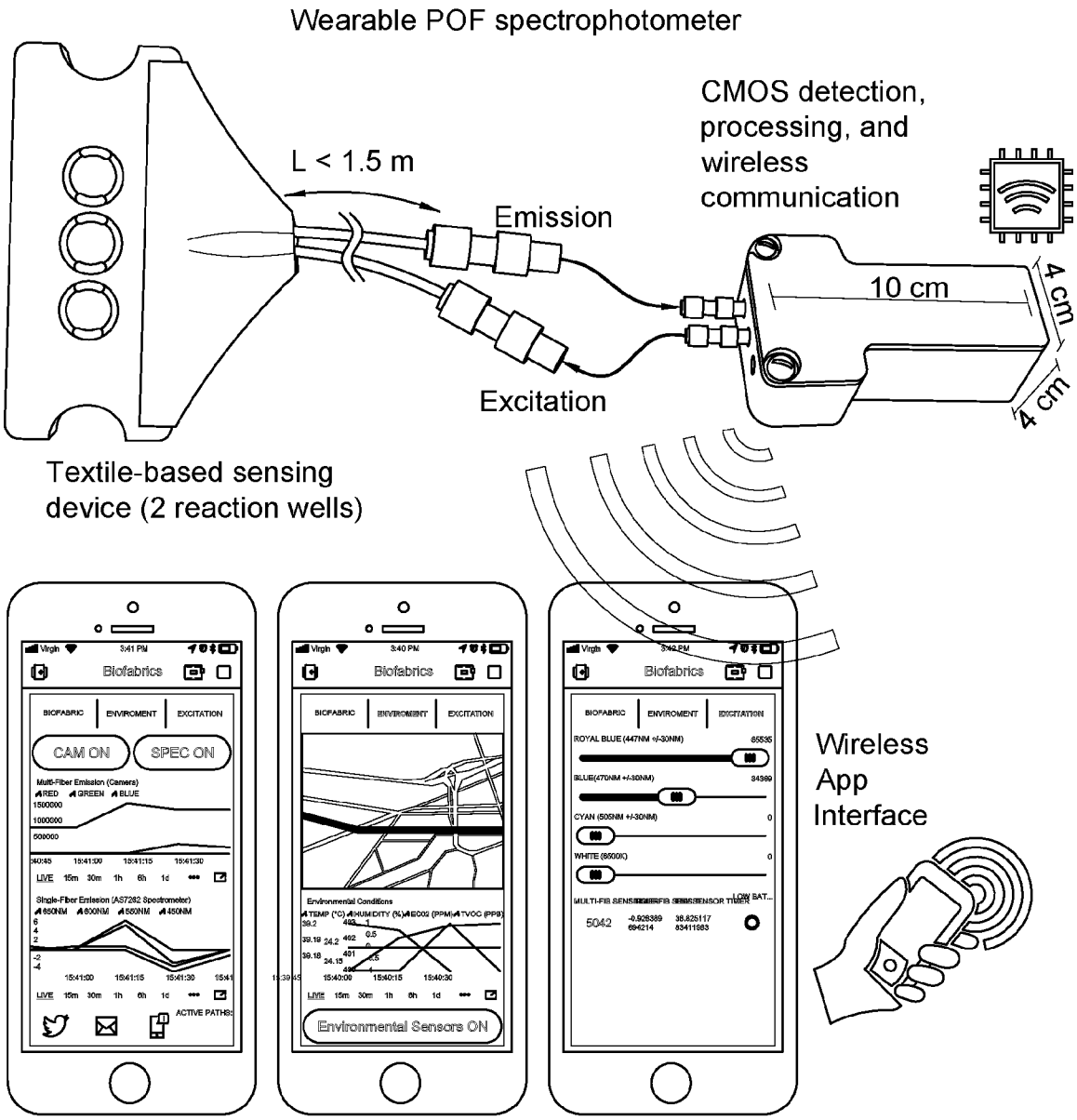

Wearable POF spectrophotometer
CMOS detection, processing, and wireless communication
L < 1.5 m
Emission
Excitation
10 cm
4 cm
4 cm
Textile-based sensing device (2 reaction wells)
Wireless App Interface
Biofabrics
BIOFABRIC
ENVIROMENT
EXCITATION
CAM ON
SPEC ON
Environmental Sensors ON

FIG. 23J

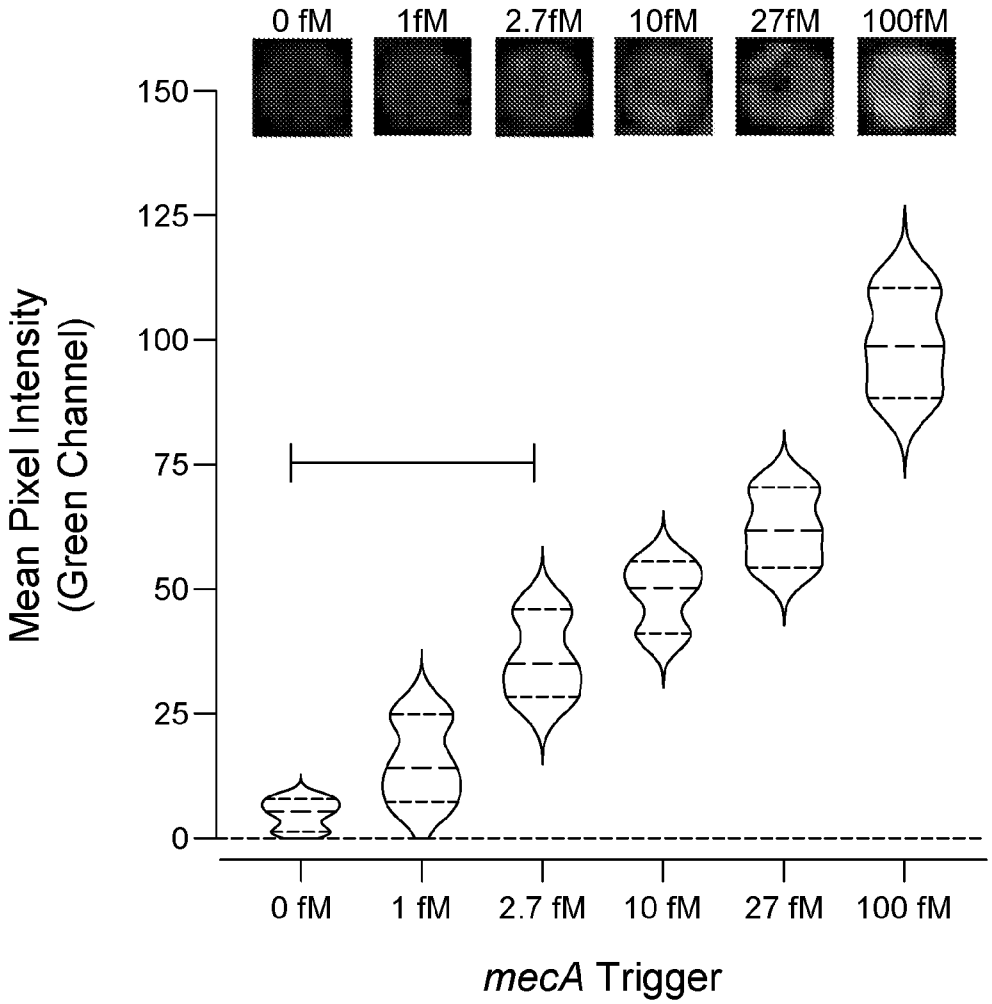

0 fM
1fM
2.7fM
10fM
27fM
100fM
150
125
100
75
50
25
0
Mean Pixel Intensity
(Green Channel)
0 fM
1 fM
2.7 fM
10 fM
27 fM
100 fM
mecA Trigger

FIG. 24

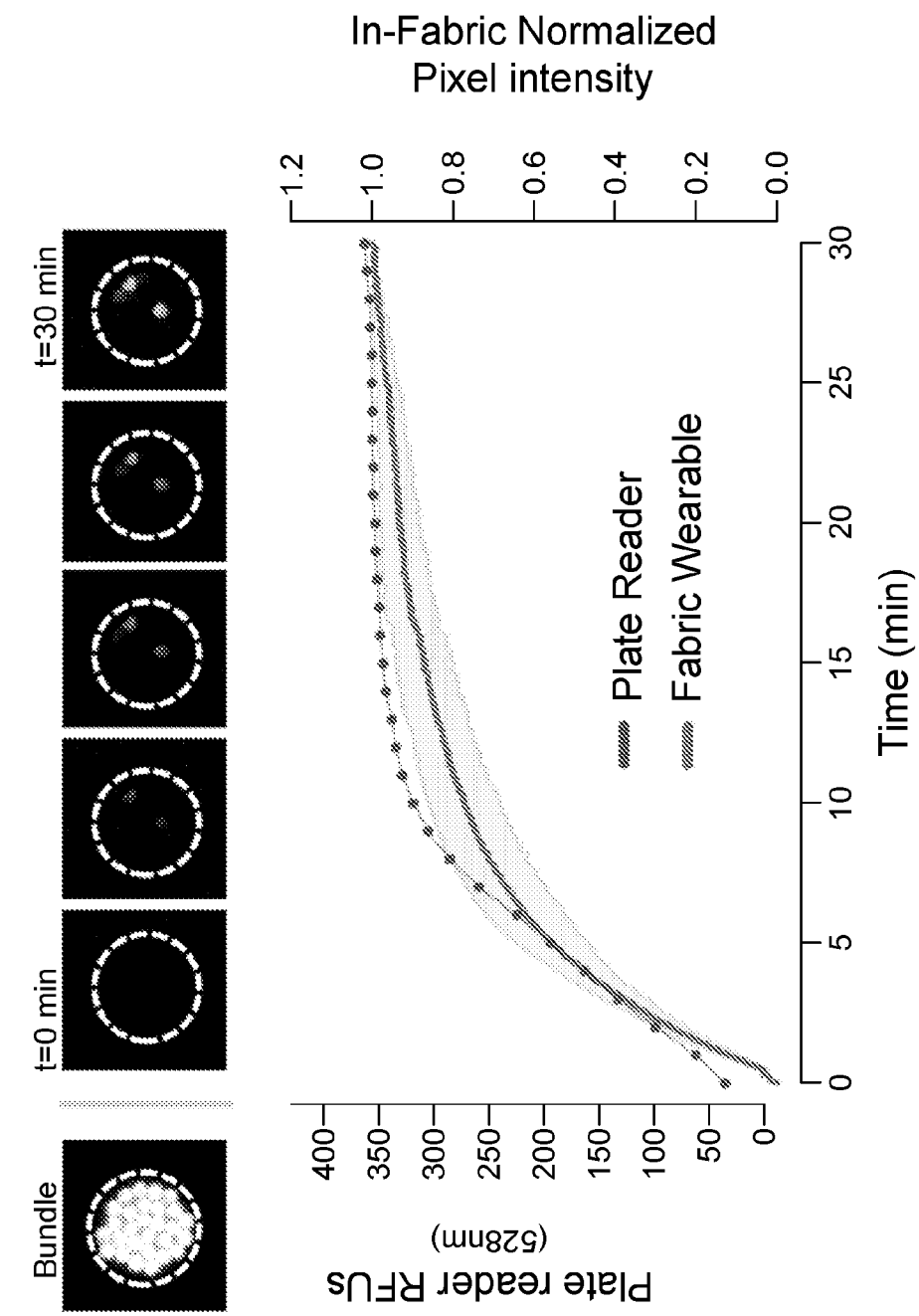

In-Fabric Normalized
Pixel intensity
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Plate Reader
Fabric Wearable
0
5
10
15
20
25
30
Time (min)
Plate reader RFUs
(528nm)
400
350
300
250
200
150
100
50
0
t=0 min
t=30 min
Bundle

FIG. 25

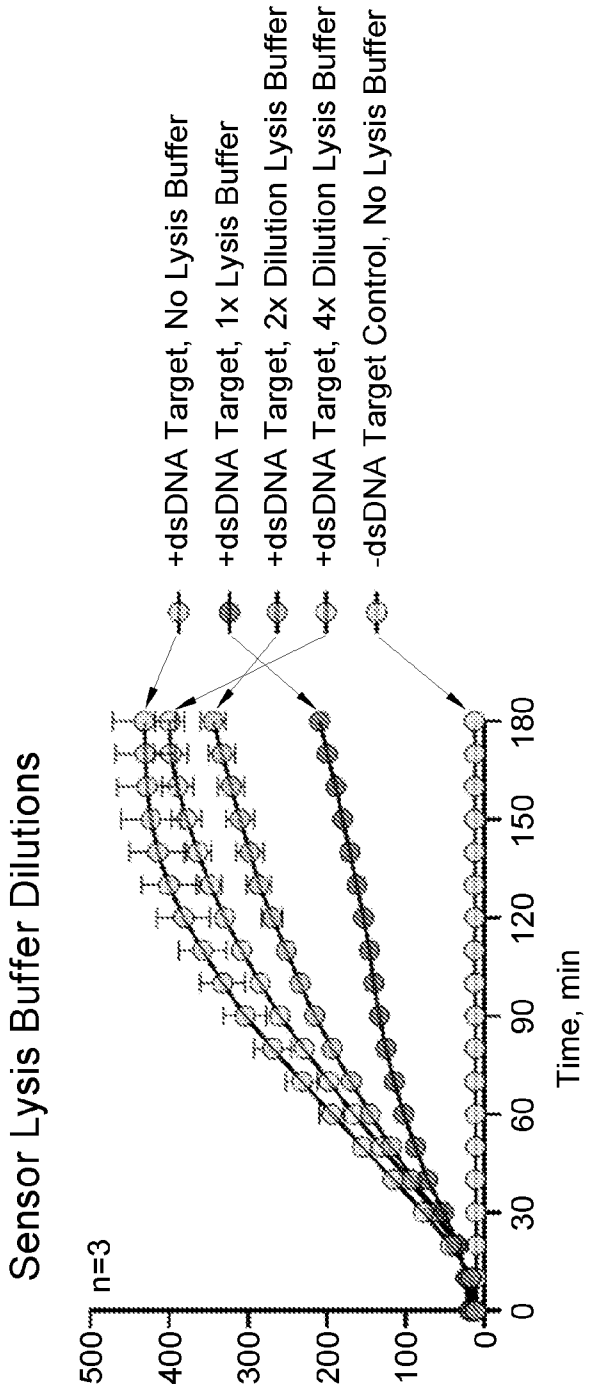

Sensor Lysis Buffer Dilutions
n=3
500
400
300
200
100
0
0
30
60
90
120
150
180
Time, min
+dsDNA Target, No Lysis Buffer
+dsDNA Target, 1x Lysis Buffer
+dsDNA Target, 2x Dilution Lysis Buffer
+dsDNA Target, 4x Dilution Lysis Buffer
-dsDNA Target Control, No Lysis Buffer

FIG. 26A

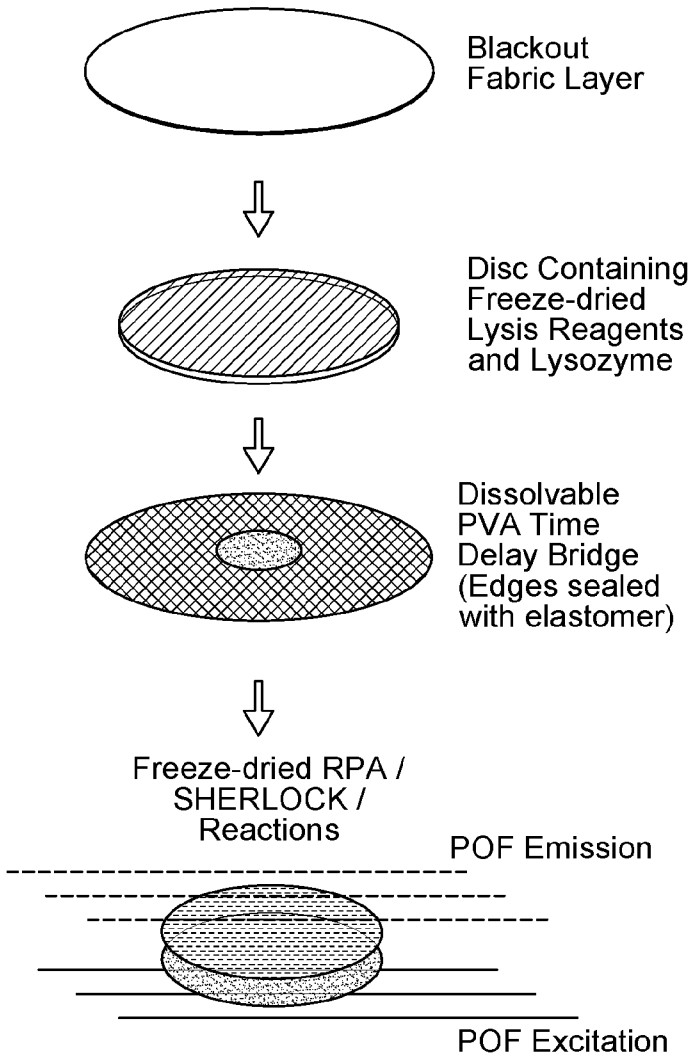

Blackout
Fabric Layer
Disc Containing
Freeze-dried
Lysis Reagents
and Lysozyme
Dissolvable
PVA Time
Delay Bridge
(Edges sealed
with elastomer)
Freeze-dried RPA /
SHERLOCK /
Reactions
POF Emission
POF Excitation

FIG. 26B

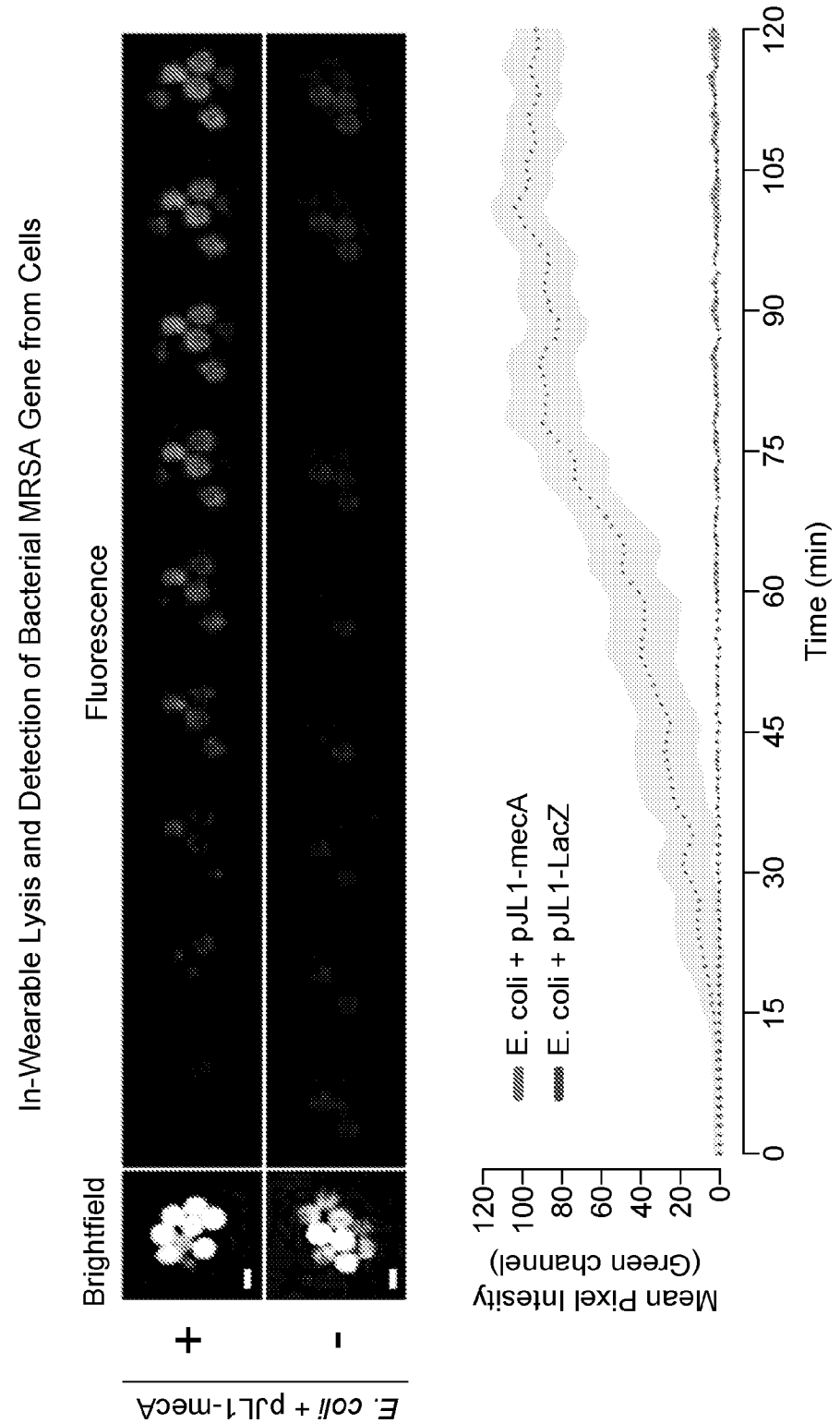

In-Wearable Lysis and Detection of Bacterial MRSA Gene from Cells
Brightfield
Fluorescence
E. coli + pJL1-mecA
+
-
E. coli + pJL1-mecA
E. coli + pJL1-LacZ
Mean Pixel Intesity
(Green channel)
120
100
80
60
40
20
0
0
15
30
45
60
75
90
105
120
Time (min)

FIG. 26C

In-wearable freeze-dried lysis reagents for autonomous sample preparation

Cas12a CoV2 Sensor in Non-Ionic Surfactants

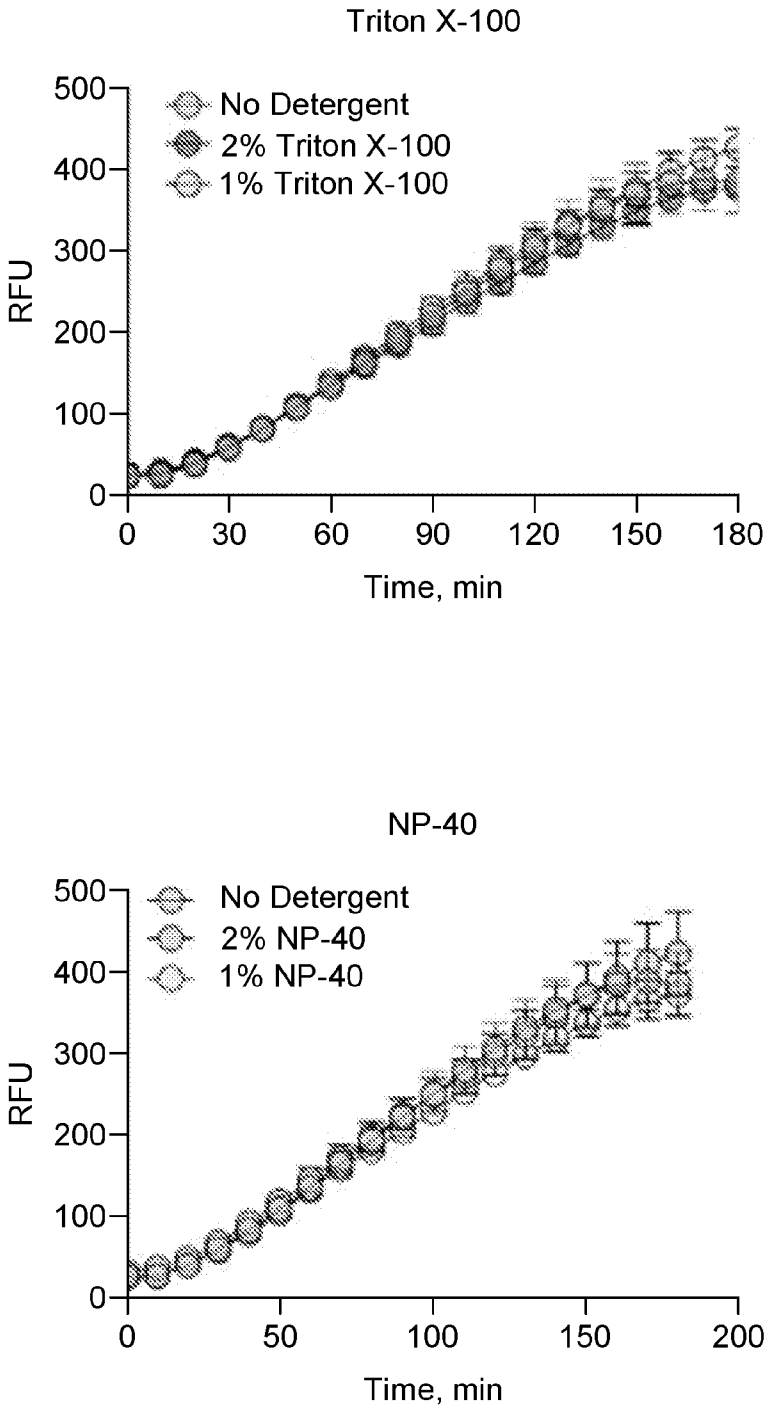
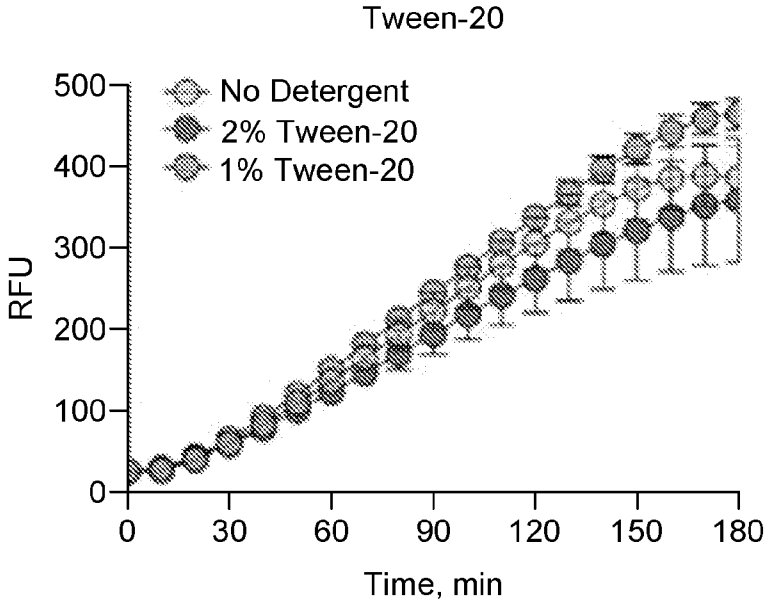


FIG. 26D



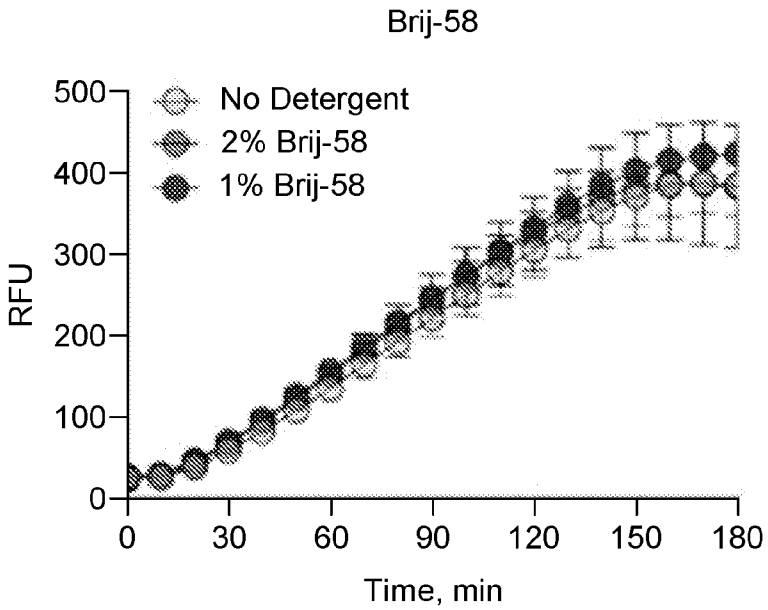
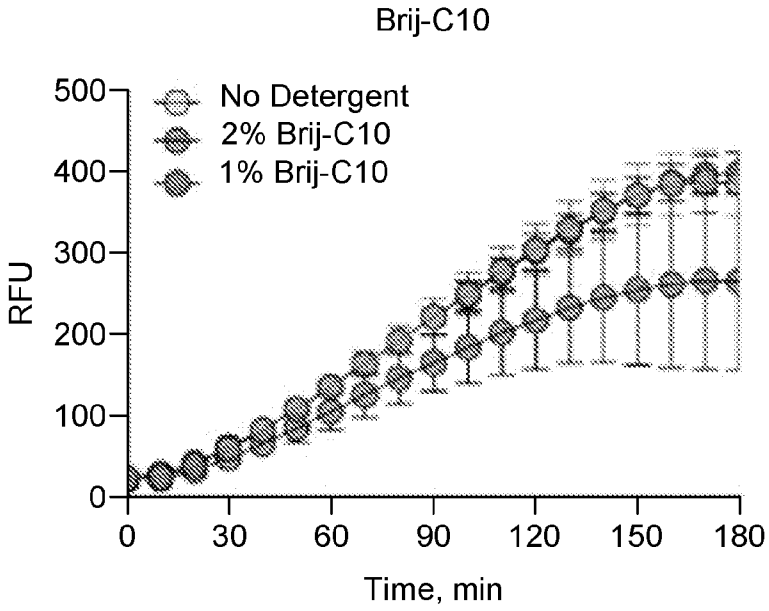


FIG. 26D (CONTINUATION)



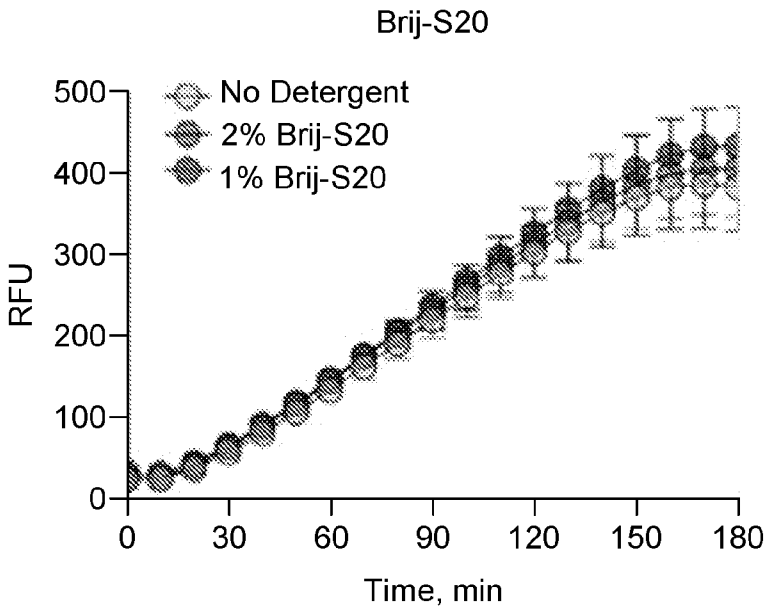


FIG. 26D (CONTINUATION)

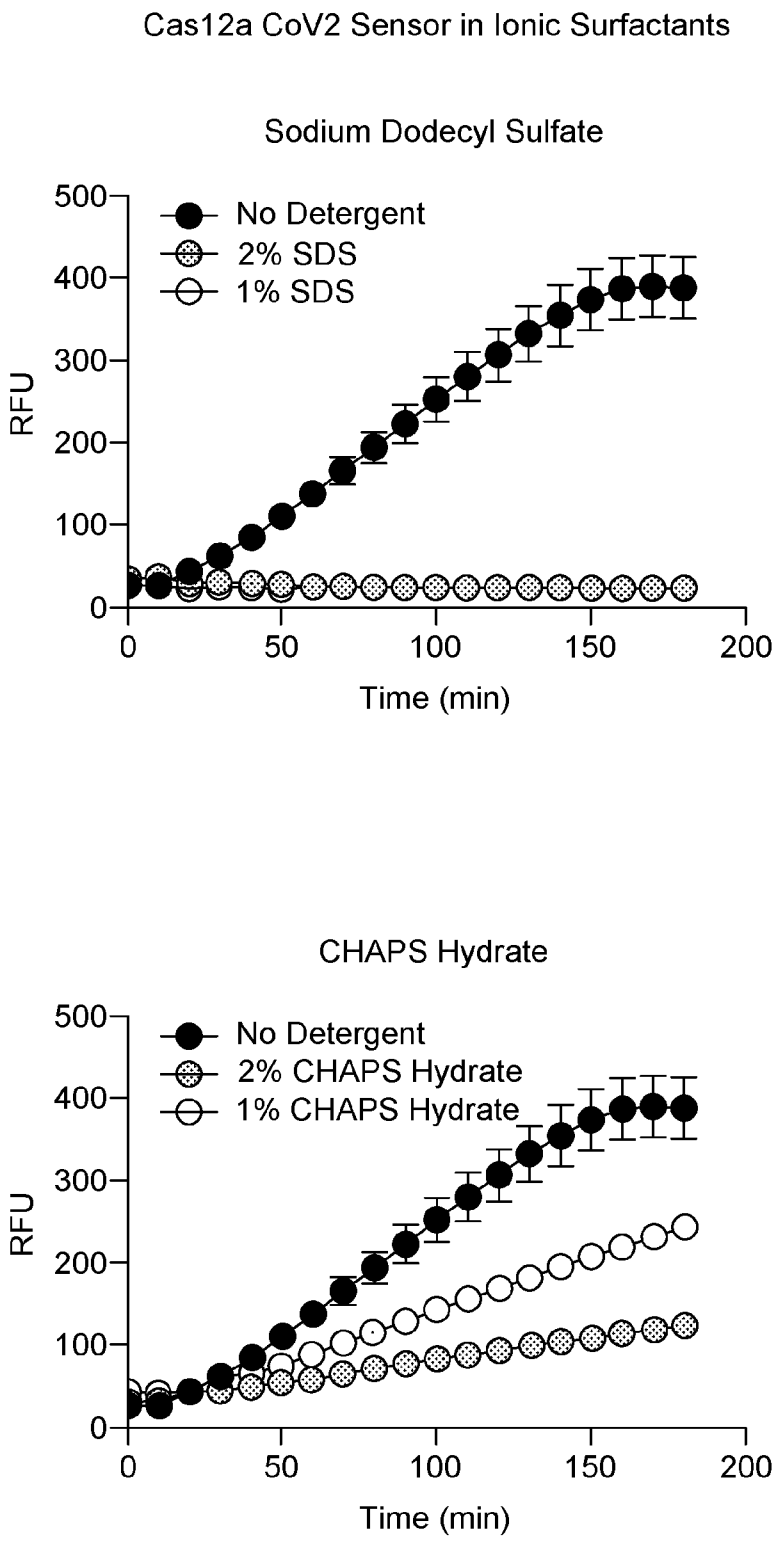

Cas12a CoV2 Sensor in Ionic Surfactants
Sodium Dodecyl Sulfate
No Detergent
2% SDS
1% SDS
RFU
Time (min)
CHAPS Hydrate
No Detergent
2% CHAPS Hydrate
1% CHAPS Hydrate
RFU
Time (min)

FIG. 26E

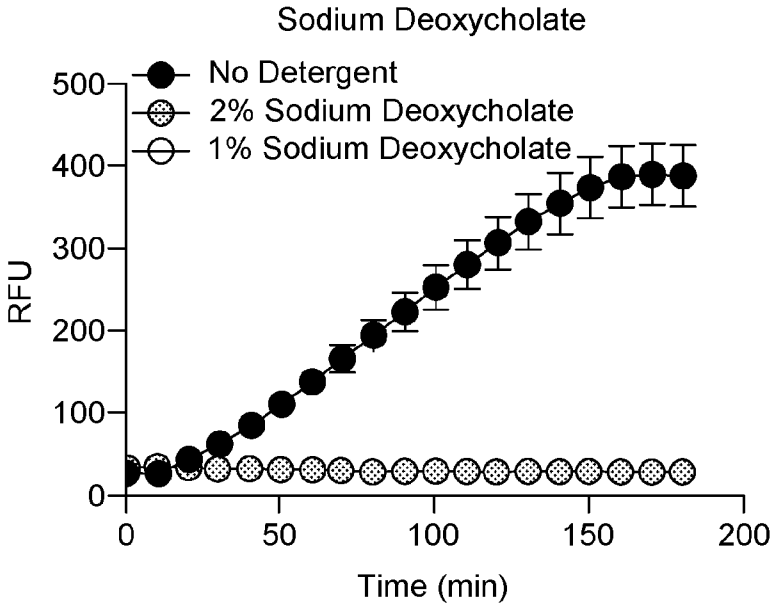


FIG. 26E (CONTINUATION)

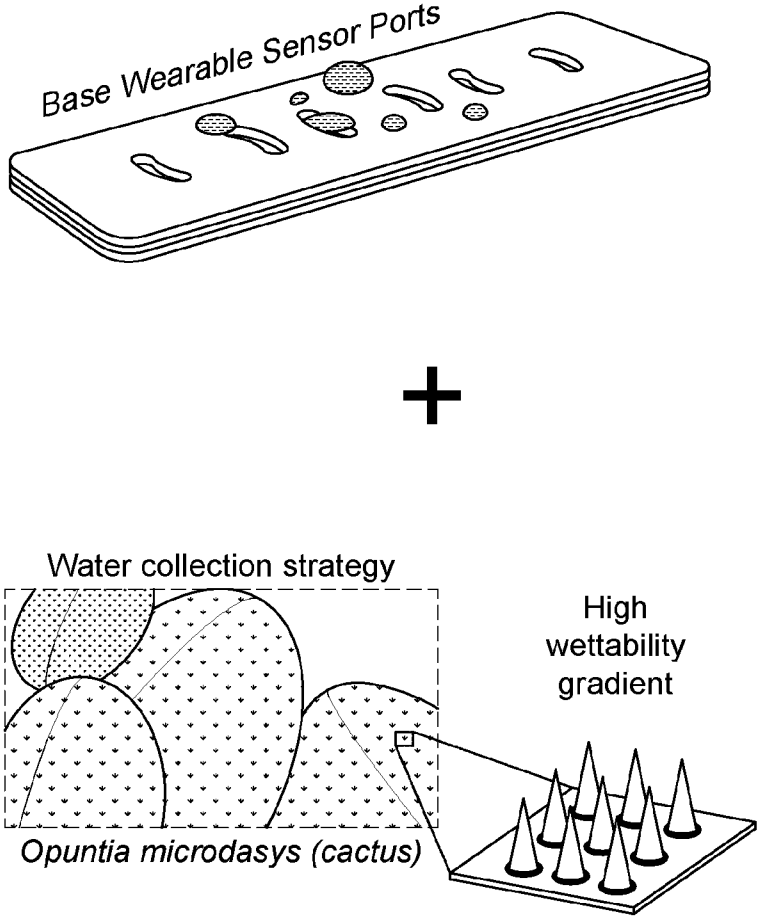

Base Wearable Sensor Ports
Water collection strategy
High
wettability
gradient
Opuntia microdasys (cactus)

FIG. 27A

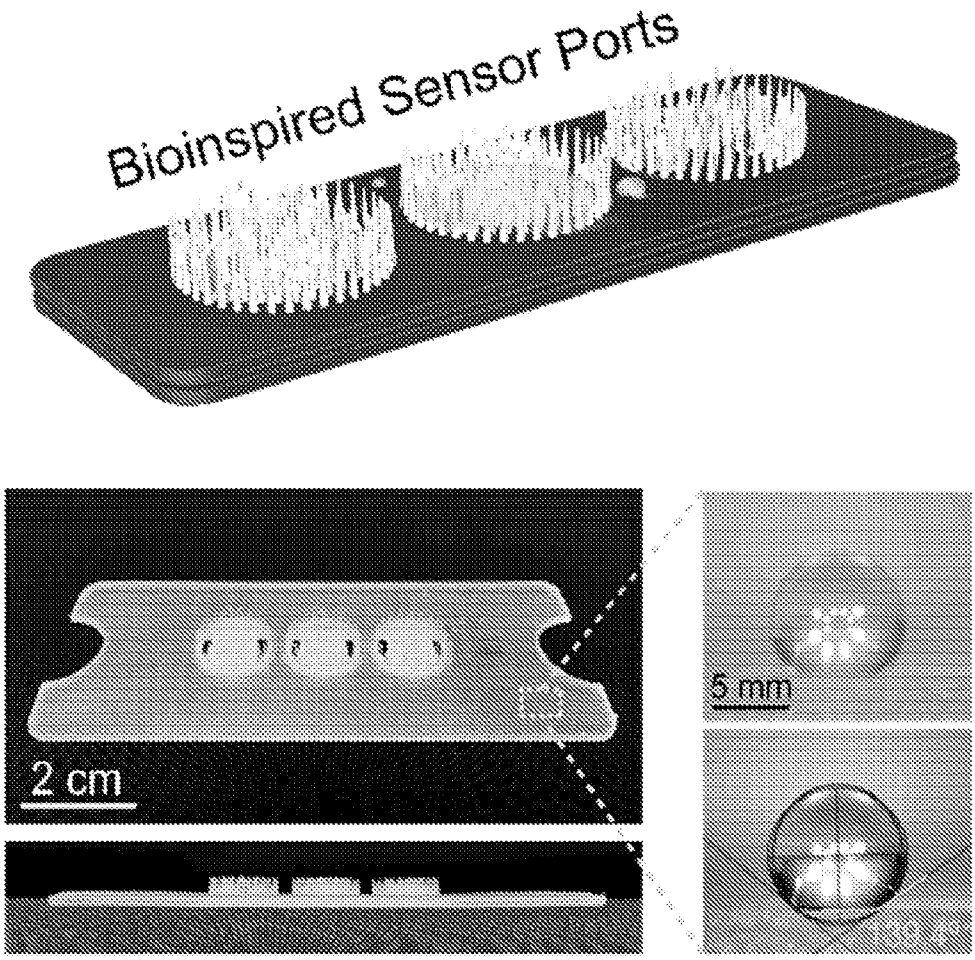


FIG. 27B

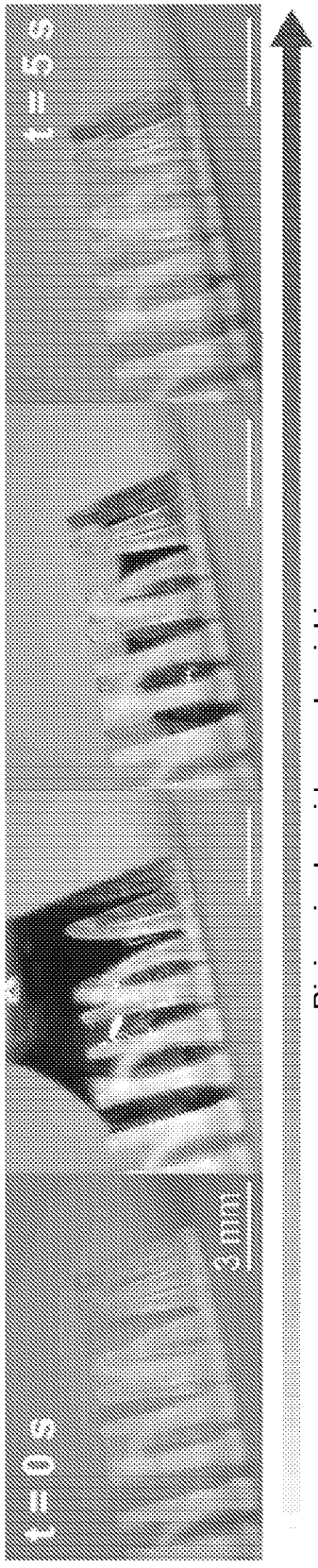


FIG. 27C

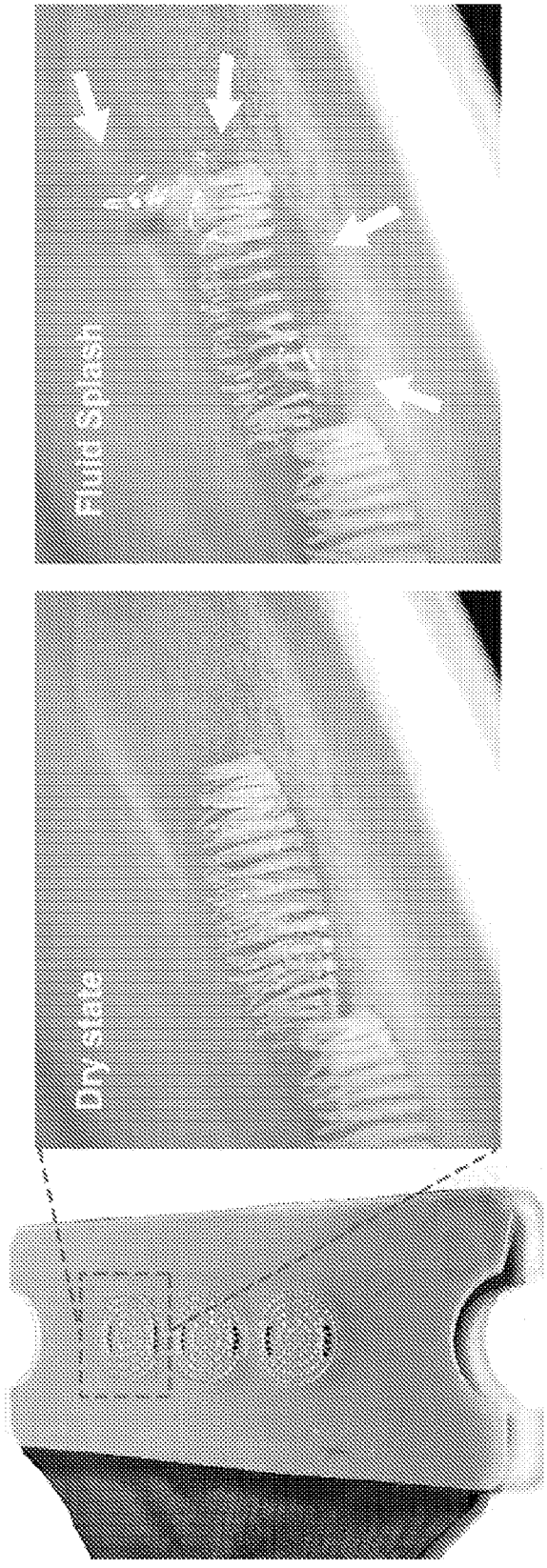


FIG. 27D

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 21/58346

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61B 5/00; A61B 5/0205; A61B 5/11 (2022.01)

CPC - A61B 5/0022; A61B 5/0082; A61B 5/0205

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Soenksen-Martinez, L. R., "Cell-Free Freeze-Dried Synthetic Biology for Wearable Biotechnology Applications", 05 February 2020 (05.02.2020), pages 1-74, entire document, especially page 29, para 1-2; page 30, para 1; page 66, para 2; Figures 2, 5, 6, 20, 33, 34, 36	1-4
A	US6117643A (Simpson et al.), 12 September 2000 (12.09.2000), entire document, especially col 3, ln 33-41, ln 57-67; col 4, ln 1-12; col 9, ln 1-22	1-4
A	US 2011/0093249A1 (Holmes et al.), 21 April 2011 (21.04.2011), entire document, especially para[0272], [0336], [0341], [0553	1-4
A	US 2018/0074080 A1 (Northwestern University), 15 March 2018 (15.03.2018), entire document, especially para[0077], [0088], [0146]	1-4

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 January 2022	MAR 11 2022
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Kari Rodriquez Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 21/58346

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 5-10, 15-47, 52-74
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
---see supplemental box---

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
claims 1-4

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 21/58346

Box III: lack of unity

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I: Claims 1-4 directed to an aqueous solution-activated sensor fabric comprising: an excitation plastic optical fiber (POF) and an emission POF combined with a porous hydrophilic material into a flat web structure, and an freeze-dried cell-free (FDCF) synthetic biological component in at least a portion of the web structure.

Group II: Claims 11-14 directed to an aqueous solution-activated sensor comprising: a chamber formed in a flexible material; a port fluidly connecting an exterior surface of the flexible material to an interior of the chamber, the port allowing an aqueous solution in contact with the exterior surface to be wicked to the interior; a first UV-Vis light-transmitting medium providing a first optical connection from the interior of the chamber to an exterior of the chamber; and freeze-dried cell-free (FDCF) synthetic biological components disposed in the chamber, the FDCF synthetic biological components being hydrated upon exposure to the aqueous solution to form rehydrated synthetic biological components formulated to provide an optical signal transmittable through the light transmitting medium responsive to the presence or absence of a triggering compound in the aqueous solution wicked to the interior of the chamber.

Group III: Claims 48-51 directed to a method for making an aqueous solution-activated sensor, the method comprising: providing a layer of a first material, a portion of a top surface of the first material defining a bottom wall of a chamber; providing a layer of a second material on the top surface of the first material, the second material including a first continuous open space on the portion of the top surface defining the bottom wall of the chamber, the second material defining a side wall of the chamber; providing a layer of a third material on a top surface of the second material, the third material including a second continuous open space disposed above the chamber, the third material defining a top wall of the chamber including a port defined by the second continuous open space; adding synthetic biological components into the chamber; and freeze drying the synthetic biological components.

The group of inventions listed above do not relate to a single general inventive concept under PCT Rule 13.1 because, under Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Group I requires a fabric comprising an excitation plastic optical fiber (POF) and an emission POF combined with a porous hydrophilic material into a flat web structure not required for Group II.

Group I requires a fabric comprising an excitation plastic optical fiber (POF) and an emission POF combined with a porous hydrophilic material into a flat web structure not required for Group III.

Group II requires a chamber formed in a flexible material; a port fluidly connecting an exterior surface of the flexible material to an interior of the chamber, the port allowing an aqueous solution in contact with the exterior surface to be wicked to the interior; a first UV-Vis light-transmitting medium providing a first optical connection from the interior of the chamber to an exterior of the chamber; the freeze-dried cell-free (FDCF) synthetic biological components being hydrated upon exposure to the aqueous solution to form rehydrated synthetic biological components formulated to provide an optical signal transmittable through the light transmitting medium responsive to the presence or absence of a triggering compound in the aqueous solution wicked to the interior of the chamber not required by group I.

Group II requires a chamber formed in a flexible material; a port fluidly connecting an exterior surface of the flexible material to an interior of the chamber, the port allowing an aqueous solution in contact with the exterior surface to be wicked to the interior; a first UV-Vis light-transmitting medium providing a first optical connection from the interior of the chamber to an exterior of the chamber; the freeze-dried cell-free (FDCF) synthetic biological components being hydrated upon exposure to the aqueous solution to form rehydrated synthetic biological components formulated to provide an optical signal transmittable through the light transmitting medium responsive to the presence or absence of a triggering compound in the aqueous solution wicked to the interior of the chamber not required by group III.

Group III requires a method for making an aqueous solution-activated sensor, the method comprising: providing a layer of a first material, a portion of a top surface of the first material defining a bottom wall of a chamber; providing a layer of a second material on the top surface of the first material, the second material including a first continuous open space on the portion of the top surface defining the bottom wall of the chamber, the second material defining a side wall of the chamber; providing a layer of a third material on a top surface of the second material, the third material including a second continuous open space disposed above the chamber, the third material defining a top wall of the chamber including a port defined by the second continuous open space; adding synthetic biological components into the chamber; and freeze drying the synthetic biological components not required by groups I or II.

Groups I-III share the technical features of an aqueous solution-activated sensor comprising freeze-dried synthetic biological components.

(see supplemental box)

(lack of unity continuation)

These shared technical features, however, do not provide a contribution over the prior art, as being anticipated by the thesis entitled "Cell-Free Freeze-Dried Synthetic Biology for Wearable Biotechnology Applications" to Martinez.

Martinez teaches an aqueous solution-activated sensor comprising freeze-dried synthetic biological components (page 32, para 1, 'Saturation reaction disks were then snap-frozen using liquid nitrogen and put in a vacuum container to be dried for 4-8 hours using an SP Scientific Freezemobile lyophilizer (SP Industries, Inc., Warminster, PA)...This design was confirmed to allow for entry of fluids such as dd-H2O, while still delaying evaporation of cell-free reaction after splash activation (Figure 8a)...A magnified view of an example activated Ebola virus DNA toehold wCF-FD-SB sensor within these chambers is shown in Figure 8b'; Figures 8a-b; wCF-FD-SB stands for wearable cell-free freeze-dried synthetic biology).

As the technical features were known in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups. Groups I-III therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.

Note:

Claims 5-10, 15-47, and 52-74 determined unsearchable because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).