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INVENTORS : Emil KARTALOV *et al.*

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EXAMINER : Russell Scott NEGIN

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ART UNIT : 1672

FOR : SIGNAL ENCODING AND DECODING IN MULTIPLEXED BIOCHEMICAL ASSAYS

MAIL STOP AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

RESPONSE TO FINAL OFFICE ACTION

Dear Sir:

In response to the Non-Final Office Action mailed **May 10, 2023**, please amend the above-identified application as follows:

Listing of Claims begin on page **2** of this paper.

Remarks begin on page **6** of this paper.

It is not believed that extensions of time or fees for net addition of claims are required beyond those that may otherwise be provided for in documents accompanying this paper. However, if additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned for under 37 C.F.R. § 1.136(a), and any fees required therefor (including fees for net addition of claims) are hereby authorized to be charged to Deposit Account No. 504561 (**Ref. No. 30KJ-330783-US4**).

LISTING OF CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application:

1.-20. (Canceled).

21. (Currently Amended) An assay that is capable of unambiguously detecting the presence or absence of each of at least **M** analytes, wherein each of said **M** analytes is encoded as a value of one component of a fluorescent signal, thereby generating a coding scheme, wherein said fluorescent signal comprises **F** wavelength components, and wherein said assay is capable of detecting the presence or absence of each of said at least **M** analytes, in any combination of presence or absence, in a single sample volume ~~without immobilization, separation, mass spectrometry, or melting curve analysis~~, based on detection of said **F** wavelength components, when **M** is greater than **F**, wherein the assay does not require the use of mass spectrometry or immobilization of said **M** analytes, and wherein the analytes are amplified in the single sample volume.

22. (Previously Presented) The assay of claim 21, wherein each of said at least **M** analytes is encoded as at least one first value in a first component of said fluorescent signal and at least one second value in a second component of said fluorescent signal.

23. (Previously Presented) The assay of claim 22, wherein said first value is an intensity or range of intensities.

24. (Previously Presented) The assay of claim 21, wherein said detecting is performed with reagents comprising hybridization probes.

25. (Previously Presented) The assay of claim 24, wherein said hybridization probes comprise one or more hybridization probes specific for different analytes and comprising an identical fluorophore or combination of fluorophores.

26. (Previously Presented) The assay of claim 21, wherein said signal is generated during a polymerase chain reaction.

27. (Previously Presented) The assay of claim 21, wherein at least one of said analytes is encoded by at least one additional value, wherein said at least one additional value is selected from the group consisting of a value from at least one additional component of said signal, a value from at least one component of an additional signal, and combinations thereof.

28. (Previously Presented) The assay of claim 24, wherein said fluorescence signal from said hybridization probes are used to construct a chromatogram.

29. (Previously Presented) The assay of claim 21, wherein at least one of said analytes comprises a polynucleotide.

30. (Previously Presented) The assay of claim 21, further comprising transmitting information concerning the presence or absence of at least one of said analytes through a computer network.

31. (Previously Presented) The assay of claim 21, wherein at least one step of said assay is performed using a computer and wherein said computer is connected to a thermal cycler.

32. (Previously Presented) The assay of claim 21, wherein said coding scheme is innately nondegenerate or non-degenerate by construction.

33. (Previously Presented) The assay of claim 22, wherein said coding scheme is made nondegenerate by enumerating every legitimate result that can be obtained from said coding

scheme, identifying each legitimate result that is degenerate, and eliminating at least one potential analyte code from said coding scheme to eliminate degeneracy.

34. (Currently Amended) A kit for detecting the presence or absence of at least M analytes by generating a single cumulative measurement, comprising analyte-specific reagents, wherein:

- a. each of said at least M analytes is encoded as a value of one component of a fluorescent signal, thereby generating a coding scheme, wherein each of said at least M analytes is represented in said coding scheme by said value, and wherein said encoding is performed in a manner that eliminates degeneracy; and
- b. each of said analyte-specific reagents, when contacted with a sample comprising its corresponding analyte, generates the value;

wherein said kit is capable of unambiguously detecting the presence or absence of each analyte of M analytes in a single sample volume, in any combination of presence or absence, and wherein the kit does not require the use of mass spectrometry or immobilization of said M analytes, and

wherein the analytes are simplified in the single sample volume.

35. (Canceled).

36. (Previously Presented) The kit of claim 34, wherein said fluorescent signal comprises F wavelength components and said kit is capable of unambiguously detecting the presence or absence of M analytes, in any combination of presence or absence, when M is greater than F .

37. (Previously Presented) The kit of claim 34, wherein said analyte-specific reagents comprise hybridization probes.

38. (Previously Presented) The kit of claim 37, wherein said hybridization probes comprise a fluorophore, and wherein said plurality of hybridization probes are specific for different analytes.

39. (Previously Presented) The kit of claim 38, wherein said number of hybridization probes in the kit is greater than M.

40. (Previously Presented) The kit of claim 34, wherein the analyte-specific reagents are each present at a different amount in the kit.

REMARKS

Claims 21-42 are pending. Claims 21 and 34 are currently amended. Support can be found throughout the Specification and at least at paragraphs [0014], [0043]-[0044], [0058], [0068], [0110], [0117], [0142], [0147]-[0148], and [0158]-[0168]. No new matter is added. Claim 35 is cancelled. In view of the amendments and following remarks, each of the presently pending claims in this application is believed to be in immediate condition for allowance. Accordingly, Applicant respectfully requests that the Examiner reconsider all outstanding rejections and allow the claims.

Claims Rejections Under 35 U.S.C. §103

Rejection of Claims 21-25, 27, 29-30 and 32-40 under 35 U.S.C. § 103 as being unpatentable over Han in view of Lin

Claims 21-25, 27, 29-30 and 32-40 are rejected under pre-AIA 35 U.S.C. § 103(a) as being unpatentable over Han et al. (Nature Biotechnology, volume 19, 2001, pages 631-635) (“Han”) in view of WO 2012/058628 [sic, WO 2012/058638] (“Lin”). Applicant respectfully disagrees for at least the reasons previously submitted.

Additionally, contrary to the Office Action, as alleged at page 4, the Han assay describes not one, but at least three steps of immobilization.

First, the Han assay, at the centrifugation step, describes that “[b]efore fluorescence measurement, the beads were cleaned by two rounds of centrifugation” (see Han at page 635). Thus, the Han assay clearly requires separating the mobile solution phase from the immobile fluorescence beads that were collecting as a pellet. This is in stark contrast to the claimed assay and kit, as amended, clearly stating that wherein the assay or kit “does **not** require ... **immobilization of said M analytes**” (emphasis added). In fact, the Specification of this Application does not mention the terms “centrifuge,” “centrifugation,” “centrifuging,” “spin,” “spinning,” and “spung” even once. Instead, the Specification of this Application clearly distinguishes the novel and non-obvious assay and kit disclosed here to not require “immobilization, separation, mass spectrometry, or melting curve analysis” (see for example, paragraphs [0005], [0033], [0039]-[0041], [0121]). Moreover, the Specification at paragraph [0155] specifically states that “[i]mportantly, the methods and compositions provided herein may

be used to detect multiple analytes by obtaining a cumulative measurement on a single solution. **No separation is necessary. The invention does not require the use of beads or a solid phase** (emphasis added). Thus, a person of ordinary skill in the art would not have considered the separation and immobilization bead technology of Han, whether alone or in combination with any cited references, to arrive at the claimed assay and kit. For at least this reason, reconsideration and withdrawal of the rejection are respectfully requested.

Second, the Han assay, at the imaging step, describes that “[t]he beads were spread and immobilized on a polylysine-coated glass slide.” It is well known to a person of ordinary skill in the art that polylysine, here, is specifically **used to attach and immobilize the fluorescence beads** to the slide (see Fisher Scientific Product No. 50-279-88 describing the poly-l-lysine coated slides to “improve the adhesion of tissues to the slide.”) Applicant respectfully points out that the specific choice of using polylysine-coated slides was **not** representative of a necessary and ordinary step for experimental observation. A person of ordinary skill in the art would have recognized that glass slides **not** coated with polylysine are more commonly available, less expensive, and are commonly used for experimental observation, including fluorescence imaging, that does not require immobilization or strong adhesion to the slides. Indeed, a person of ordinary skill in the art would have understood that the Han assay (1) specifically chose a polylysine-coated glass slide, as opposed a non-coated slide to immobilize the fluorescence beads; (2) specifically covalently attached streptavidin molecule to the surface of the beads, to enhance the immobilization of the beads (see Han at for example page 635); and (3) specifically packed the nonspecific binding sites on the surface of the beads with the protein BSA to enhance immobilization of the beads to the polylysine-coated glass slide. This, again, is in stark contrast to the claimed assay and kit, clearly stating that it “does **not** require ... **immobilization of said M analytes**” (emphasis added). In fact, the Specification of this Application does not mention the terms “slide,” “coat,” “coated,” and “polylysine,” even once. Thus, a person of ordinary skill in the art would not have arrived at the claimed assay or kit in view of Han, whether alone or in combination with any cited references. For at least this reason, reconsideration and withdrawal of the rejection are respectfully requested.

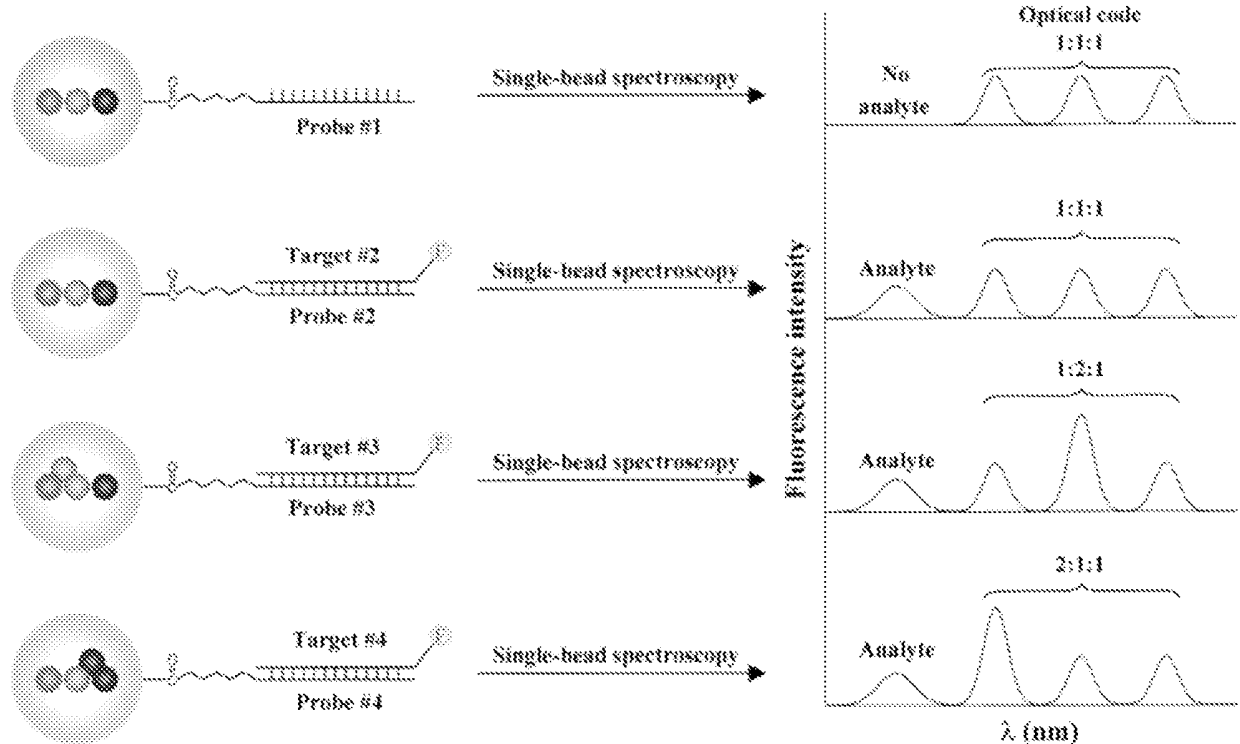
Third, a person of ordinary skill in the art would have viewed attaching the target oligonucleotides of Han to QD-tagged beads through cross-linked probes would be a step of

immobilization as the term is used in the Specification. Indeed, the term “immobilization” as used in the Specification requires no additional definition and the implications of using the term “immobilization,” requires no additional explanation. A person of ordinary skill in the art would have understood “immobiliz[ing] a target gene onto insoluble [bead] particles” have been described in the context of PCR methods. See, for example, Andreadis et. al, Nucleic Acids Research, volume 28, e5 i-viii, 1999 entitled “Use of immobilized PCR primers to generate **covalently immobilized DNAs** for *in vitro* transcription/translation reactions” (“Andreadis”), where the authors describe covalently attaching, through chemical crosslinkers, two primers to a bead particle during or after DNA synthesis to achieve immobilization. The Abstract of Andreadis, for example, states as follows, with emphasis added:

We have developed a novel biochemical method to simultaneously amplify and **immobilize** a target gene onto **insoluble particles** using PCR. This method employs the covalent attachment of one of two PCR primers to a particle surface either directly during DNA synthesis of the primer or post-DNA synthesis, through the use of chemical crosslinkers. **Immobilization** of the target gene can be achieved directly during PCR amplification, with one **bead-bound** primer and one soluble primer. Alternatively, this can be achieved post-PCR, through covalent attachment of a chemically modified primer incorporated into the amplicon to **an activated particle**. All of the **immobilized DNA templates** containing appropriate regulatory regions were fully competent for transcription and translation reactions and several could be re-used in serial reactions. The most successful strategy utilized **amino-silanized controlled pore glass beads**, which were coupled to phosphorylated primers using carbodiimide chemistry. These **bead-bound** primers were used during PCR to generate attached DNA templates that could be collected and re-used for at least seven sequential transcription reactions without significant loss in efficiency. This method has also been successfully applied to the amplification, transcription and translation of multiple DNA templates using a single, **immobilized** primer. The combined PCR-based amplification/**immobilization** method was shown to be more durable than post-PCR chemical **immobilization** and affords the convenience of performing sequential PCR amplification, transcription and translation reactions in a single tube.

Thus, a person of ordinary skill in the art would have understood that binding of the target to the probe oligos and conjugating the probe oligos “to the beads by cross-linking” in Han would have

immobilized the target analyte, as the term “immobilization” is used in the Specification of this Application and understood by persons of ordinary skill in the art. Figure 5 of Han clearly show immobilization of the target analyte on the nanobeads.



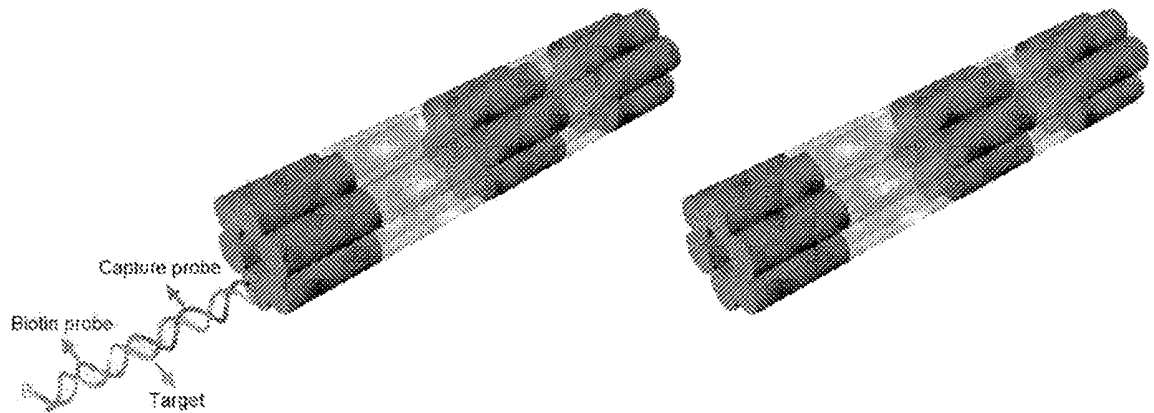
(Reproduced from Han at Figure 5).

For at least this reason, a person of ordinary skill in the art would not have arrived at the claimed assay or kit in view of Han, whether alone or in combination with any cited references.

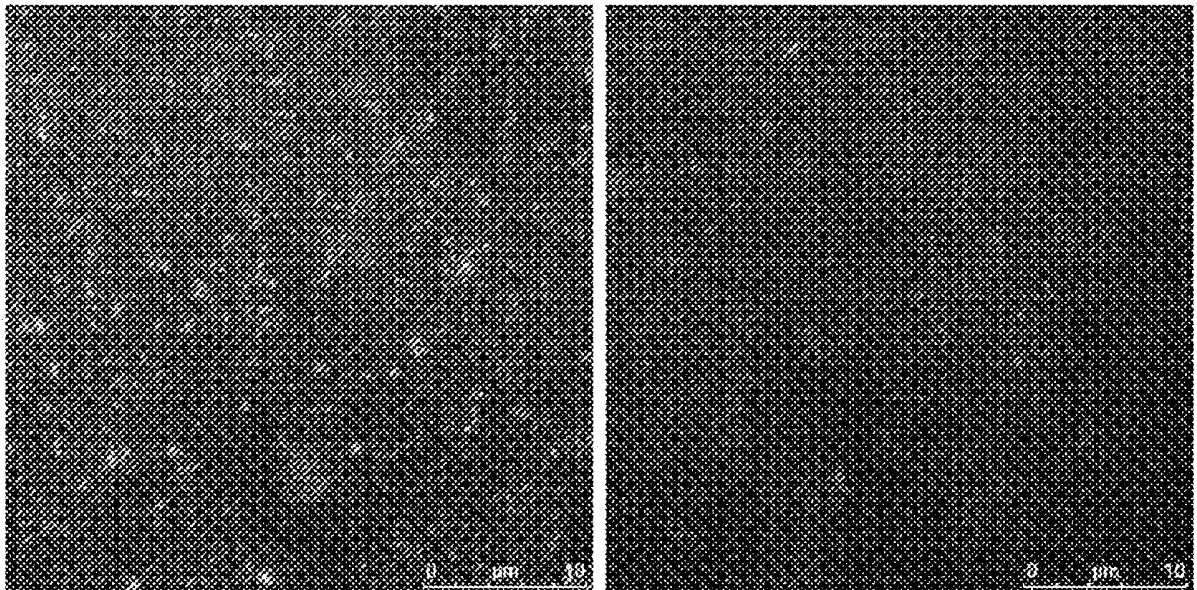
Reconsideration and withdrawal of the rejection are respectfully requested.

Lin does not cure the deficiencies in the teachings of Han, whether alone or in combination with any other cited reference. Like Han, Lin discloses immobilization of target analyte onto nanotubes (see Lin at FIG. 4A, reproduced below). Also like Han, Lin describes “immobiliz[ation]” of “nano-barcodes” on “streptavidin coated glass cover-slips (see Lin at FIG. 4B, reproduced below).

A



B



Thus, neither Han nor Lin, whether alone or in combination, would have led one of ordinary skill in the art to arrive at the claimed assay or kit, which does not require immobilization of the analytes, without undue experimentation. The combination of Han and Lin fails to teach or suggest all elements of claims 21 and 34, as amended. Claims 22-25, 27, 29-30, 32-34, and 36-40, depend directly or indirectly on claim 21 or claim 34, and incorporate the limitations therein. Accordingly, Applicant respectfully requests reconsideration and withdrawal of the rejection of claims 22-25, 27, 29-30, 32-34, and 36-40 under 35 U.S.C. § 103.

Moreover, claims 21 and 34, as amended recite “and wherein the analytes are amplified in the single sample volume.” Han and Lin, whether alone or in combination, fail to teach or

even suggest an assay or a kit wherein the analytes are amplified in the single sample volume. Applicant respectfully points out neither Han nor Lin mention “PCR” or “amplification” even once. Thus, for at least these reasons, the claims of this Application are novel and non-obvious over Han and Lin, whether alone or in combination with any other cited reference. Applicant respectfully requests reconsideration and withdrawal of the rejection of claims 22-25, 27, 29-30, 32-34, and 36-40 under 35 U.S.C. § 103.

Rejection of Claims 26 and 31 under 35 U.S.C. § 103 as being unpatentable over Han in view of Lin, and in further view of Albani

Claims 26 and 31 are rejected under pre-AIA 35 U.S.C. § 103(a) as being unpatentable over Han in view of Lin and in further view of US Published Application No. 2006/0039918 (“Albani”). Applicant respectfully disagrees for at least the reasons previously submitted.

Additionally, as explained above, the Han assay describes at least the three steps of immobilization. Thus, a person of ordinary skill in the art would not have arrived at the claimed assay in view of Han, whether alone or in combination with any cited references. Lin does not cure the deficiencies in the teachings of Han, whether alone or in combination with any other cited reference. Albani, which discloses the use of HLA pan DR-binding peptides to modulate, block, or inhibit immune responses to effect treatment of immune-mediated diseases and conditions, such as inflammatory and autoimmune diseases, cancer, and microbial infections (see Albani at abstract). Thus, for at least these reasons reconsideration and withdrawal of the rejection are respectfully requested.

Moreover, claim 21, as amended recite “and wherein the analytes are amplified in the single sample volume.” As discussed above, Han and Lin, whether alone or in combination, fail to teach or even suggest an assay or a kit wherein the analytes are amplified in the single sample volume. Albani does not cure the deficiencies in the teachings of Han and Lin, whether alone or in combination with any other cited reference. In fact, Albani only gloss over the use of existing TaqMan qPCR technology and the store-purchased Perkin-Elmer ABI PRISM 7700 thermal cycler to conduct standard analysis and quantifications of mRNA levels. Applicant respectfully request Examiner to explain how the analyte, having being bound to the nanobeads of Han, the nanotubes of Lin, or the peptides of Albani could be “amplified in the single sample volume”, without undue experimentation. Thus, for at least these reasons, the claims of this Application

are novel and non-obvious over Han, Lin, and Albani, whether alone or in combination with any other cited reference. Applicant respectfully requests reconsideration and withdrawal of the rejection of claims 26 and 31 under 35 U.S.C. § 103.

Rejection of Claim 28 under 35 U.S.C. § 103 as being unpatentable over Han in view of Lin and Hardin

Claim 28 are rejected under pre-AIA 35 U.S.C. § 103(a) as being unpatentable over Han in view of Lin and U.S. Patent Publication No. 2010/0317005 (“Hardin”). Applicant respectfully disagrees for at least the reasons previously submitted.

Additionally, as explained above, Han assay describes at least the three steps of immobilization. Thus, a person of ordinary skill in the art would not have arrived at the claimed assay in view of Han, whether alone or in combination with any cited references. Lin does not cure the deficiencies in the teachings of Han, whether alone or in combination with any other cited reference. Hardin, which describes its invention as “[t]he present invention provides a method for screening kinases including the step of providing one **immobilized** substrate or a plurality of immobilized substrates on a support” (see Hardin at least at paragraph [0070]) also fails to cure the deficiencies of Han and Lin. Thus, for at least these reasons reconsideration and withdrawal of the rejection are respectfully requested.

Moreover, claim 21, as amended recite “and wherein the analytes are amplified in the single sample volume.” As discussed above, Han and Lin, whether alone or in combination, fail to teach or even suggest an assay or a kit wherein the analytes are amplified in the single sample volume. Albani does not cure the deficiencies in the teachings of Han and Lin, whether alone or in combination with any other cited reference. Applicant respectfully request Examiner to explain how the analyte, having being bound to the nanobeads of Han or the nanotubes of Lin could be combined with the labeled nucleotide triphosphates of Albani to arrive at the claimed embodiment of “wherein the analytes are amplified in the single sample volume”, without undue experimentation. Thus, for at least these reasons, the claims of this Application are novel and non-obvious over Han, Lin, and Hardin, whether alone or in combination with any other cited reference. Applicant respectfully requests reconsideration and withdrawal of the rejection of claim 28 under 35 U.S.C. § 103.

Obviousness-Type Double Patenting

Rejection of Claims 21, 23, 26, 27, and 29-34

Claims 21, 23, 26, 27, and 29-34 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1, 3, 7-8, 11-12, 15-17 and 20, respectively, of U.S. Patent No. 8,838,394. Applicant respectfully disagree for at least the reasons previously submitted. Moreover, for at least the reason that obviousness-type double patenting cannot be finally determined until there is allowable subject matter, Applicant requests deferral of this issue until there is an indication of allowable claims.

Rejection of Claim 21, 26, 30-31

Claim 21, 26, 30-31 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1, 4-5, and 9 of U.S. Patent No. 10,068,051. Applicant respectfully disagree for at least the reasons previously submitted. Moreover, for at least the reason that obviousness-type double patenting cannot be finally determined until there is allowable subject matter, Applicant requests deferral of this issue until there is an indication of allowable claims.

CONCLUSION

If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided. Prompt and favorable consideration of this Response is respectfully requested. The Commissioner is authorized to charge any underpayment or credit any overpayment to Deposit account No. 50-4561 (Attorney Docket No. 88DG-330783-US4)

Respectfully submitted,

SHEPPARD MULLIN RICHTER & HAMPTON LLP

By / Jesse A. Salen /
Jesse A. Salen
Registration No.: 72,209

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12275 El Camino Real, Suite 100
San Diego, CA 92130-4092
Telephone: (858) 720-8900
Facsimile: (858) 509-3691