UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Ex parte JAMES W. JACOBSON, JENNIFER L. BURROUGHS, and KERRY G. OLIVER

Appeal 2007-2727
Application 09/956,857
Technology Center 1600

Decided: May 30, 2008

Before, TONI R. SCHEINER, DONALD E. ADAMS, and DEMETRA J. MILLS, *Administrative Patent Judges*.

MILLS, *Administrative Patent Judge*.

DECISION ON APPEAL

This is an appeal under 35 U.S.C. § 134. The Examiner has rejected the claims for anticipation and obviousness. We have jurisdiction under 35 U.S.C. § 6(b). We reverse.

The following claims are representative.

1. A method for detection of different reactive sites on at least one analyte, the method comprising:

(1) providing a population of microspheres, wherein each of the microspheres carries different reactants capable of reacting respectively with the different reactive sites, wherein the at least one analyte comprises a nucleic acid molecule, wherein the different reactive sites comprise one or more alleles of a locus on the nucleic acid molecule, and wherein the different reactants comprise one or more fluorescently-labeled nucleic acid probes respectively specific for the one or more alleles;

(2) allowing the different reactants and the different reactive sites to react, thereby forming reactant-reactive site pairs on the microspheres, wherein different reactant-reactive site pairs are detectably distinguishable from each other by their fluorescence intensity, and wherein the different reactant-reactive site pairs are not detectably distinguishable from each other by their fluorescence wavelength; and

(3) detecting the fluorescence intensity of the reactant-reactive site pairs formed on one of the microspheres simultaneously, whereby the presence or absence of each of the different reactive sites on the at least one analyte is determined based on the fluorescence intensity of the reactant-reactive site pairs.

7. The method of Claim 1, wherein the fluorescence intensity of the reactant-reactive site pairs is detected using flow cytometry.

15. The method of Claim 14, wherein the genetic locus is the human BRCAl gene.

16. The method of Claim 14, wherein the nucleic acid molecules comprise a mixture of nucleic acid molecules from more than one organism.

40. The method of claim 29, wherein the analyte is a human chorionic gonadotropin (hCG) related molecule, wherein the different reactive sites comprise a alpha-subunit or a variant thereof or a beta-subunit or a variant thereof, and wherein the different reactants comprise a respective antibody.

Cited References

Gunderson	US 2003/0215821	Nov. 20, 2003
Nolan	US 6,287,766 B1	Sept. 11, 2001
King	US 5,622,829	Apr. 22, 1997
Chandler	WO/97/14028	Apr. 17, 1997

Iannone et al., "Multiplexed Single Nucleotide Polymorphism Genotyping by Oligonucleotide Ligation and Flow Cytometry," *Cytometry*, Vol. 19, pp. 131-140 (2000)

Grounds of Rejection

1. Claims 1, 9, 10, 13-14, 17, 22-24, 29, 31, 33, 36-39, 41-44 and 46 stand rejected under 35 U.S.C. § 102(e) as anticipated by Gunderson.

2. Claim 40 stands rejected under 35 U.S.C. § 103 as obvious over Gunderson in view of Chandler.

3. Claims 7, 21, 35 and 45 stands rejected under 35 U.S.C. § 103 as obvious over Nolan in view of Gunderson.

4. Claim 16 stands rejected under 35 U.S.C. § 103 as obvious over Iannone in view of Gunderson.

5. Claim 15 stands rejected under 35 U.S.C. § 103 as obvious over Gunderson in view of Nolan or Iannone and in further view of King.

DISCUSSION

Background

"Often, there is a need to perform two or more different assays on the same sample, often in a single vessel and at about the same time. Such assays are known in the art as multiplex or multiplexed assays." (Spec. 1.) "Detection of nucleotide mutations and polymorphisms is central to the modern science of molecular genetics. For example, allelic discrimination detects different forms of the same gene that differ by nucleotide substitution, insertion, or deletion. In many cases, individuals affected by a given disease display extensive allelic heterogeneity." (Spec. 1.)

"A preferred embodiment[ ] of the invention involves a method for determining an allele zygosity of a nucleic acid in a sample. A set of microspheres are first prepared, each microsphere carries an equal mixture of the two nucleic acid probes for each of two alleles, A and B. These two probes are distinguishably labeled. Allele probe hybridization is then performed, forming allele/probe pairs. Finally, signal strength for each allele/probe pair is measured, and allele zygosity is then determined according to the signal strength. If the zygosity is AA, then only probe A's signal is detected. If the zygosity is BB, only B probe signal is detected. If the zygosity is AB, then an equal strength of A and B probe signals are detected. An example of such a method is depicted in Figure 1." (Spec. 10-11.) Fig. 1 shows "a dual reporter read-out assay using direct hybridization, the fluorescence intensity of heterozygous (A/B) allele is half-way between the respective fluorescence intensities of homozygous (A/A and B/B) alleles." (Spec. 8.) "What is important and innovative, is that a single set of

microspheres carrying a substantially equal mixture of the nucleic acid probes for each of said alleles can provide substantially the same information that previously required two or even three sets of microspheres, one for each of homozygous alleles and one for a heterozygous allele." (Spec. 11.) "Further, the measuring step of the fluorescence signal comprises measuring either fluorescence intensity or fluorescence color or other well known means of light differentiation." (Spec. 11.) Specially designed flow cytometry apparatus for multiplex assays are known in the art. (Spec. 4.)

Anticipation

1. Claims 1, 9, 10, 13-14, 17, 22-24, 29, 31, 33, 36-39, 41-44, and 46 stand rejected under 35 U.S.C. § 102(e) as anticipated by Gunderson.

The Examiner finds that

> Gunderson teaches detection of a plurality of nucleic acids, the method comprising:
>
> (1) providing a population of microspheres wherein each of the microspheres carries a plurality of different reactants capable of reacting respectively with a plurality of different reactive sites … (2) allowing different reactants to react with different reactive sites on the analyte to form one or more reactant-reactive site pairs which are detectably distinguishable from each other and wherein the analyte is a nucleic acid and the different reactive sites are one or more alleles of a locus on a target nucleic acid molecule and whereby the different reactants are one or more fluorescently labeled nucleic acid probes respectively for the one or more alleles … [T]he invention provides compositions and methods for the detection (and optionally quantification) of differences or variations of sequences (e.g. SNPs) using bead arrays for the detection of the

> differences.", (3) detecting the reactant-reactive site pairs formed, whereby the presence or absence of each of the plurality of reactive, sites on the analyte is determined.

(Ans. 4.)

In particular, Gunderson teaches that if a set of four different bases is used, all four probes are differently labeled and are attached to a single bead. (Gunderson 27: ¶312.) Gunderson further teaches that fluorescence intensity may be used to distinguish between alleles and between homozygosity and heterozygosity. (Gunderson 35: ¶416.)

Appellants contend that Gunderson "does not disclose allowing different reactants carried on a microsphere to react respectively with different reactive sites on an analyte to form reactant-reactive site pairs which are distinguishable by their fluorescence intensity but not their fluorescence wavelength" as claimed (App. Br. 6.)

The Examiner responds, arguing that Gunderson teaches

> [i]n paragraph 0416 it is clear that only one fluorescent label is used and that relative fluorescence intensity is used to distinguish the alleles as well as homozygous and heterozygous samples. Therefore, when Gunderson teaches that the alleles may be distinguished by fluorescence intensity at paragraph 0416 and when Gunderson teaches that multiple probes may be placed on a single bead at paragraph 0312, these two teachings are not in conflict. The ordinary practitioner reading Gunderson would recognize that Gunderson simply requires spatial localization to exist for the fluorescent intensity analysis. This spatial localization could occur at different sites on the bead and Gunderson expressly teaches that multiple probes may be placed on a single bead.

(Ans. 17.)

The standard under § 102 is one of strict identity. "Under 35 U.S.C. § 102, every limitation of a claim must identically appear in a single prior art reference for it to anticipate the claim." *Gechter v. Davidson*, 116 F.3d 1454, 1457 (Fed. Cir. 1997). "Every element of the claimed invention must be literally present, arranged as in the claim." *Richardson v. Suzuki Motor Co., Ltd.,* 868 F.2d 1226, 1236 (Fed. Cir. 1989). We agree with Appellant and do not find the Examiner has provided evidence in the prior art of "allowing different reactants carried on a microsphere to react respectively with different reactive sites on an analyte to form reactant-reactive site pairs which are distinguishable by their fluorescence intensity <u>but not their fluorescence wavelength</u>" [Emphasis added.] (App. Br. 14).

Appellants argue that in Gunderson,

> [m]olecular beacon probes can have spectrally resolvable signals, all four probes (if a set of four different bases is used) differently labelled are attached to a single bead. (Gunderson – paragraph 0312). Therefore, Gunderson teaches that different reaction products can be detected on a single bead based on differences in the spectral response (i.e., wavelength of fluorescence) emitted probes. In addition, Gunderson states that "This reaction composition allows a biallelic marker to be ratiometrically scored; that is, the intensity of the two labels in two different 'color' channels on a single substrate is compared." (Gunderson – paragraph 0334). Therefore, Gunderson teaches that alleles can be detected based on different colors or wavelengths emitted by different labels. As such, Gunderson teaches that different reactant-reactive site pairs are distinguishable based at least in part on fluorescence wavelength.

(App. Br. 7.)

While Appellants admit that Gunderson teaches that "the identity of the alleles can be determined from the fluorescence wavelength and intensity emitted by the different molecular probes", the Examiner has failed to explain where Gunderson teaches that different reactive site pairs can be distinguished "by their fluorescence intensity but not their fluorescent wavelength" (App. Br. 7). While the Examiner argues that the ordinary practitioner reading Gunderson would recognize that Gunderson simply requires spatial localization to exist for the fluorescent intensity analysis, and argues that this spatial localization could occur at different sites on the same bead (Ans. 17-18), such a conclusion is not supported by the evidence of record.

Furthermore, the Examiner's claim analysis on pages 13-16 of the Answer fails to identify a teaching in Gunderson wherein different reactants carried on a microsphere "react respectively with different reactive sites on an analyte to form reactant-reactive site pairs which are distinguishable by their fluorescence intensity but not their fluorescence wavelength."

In view of the above, we find the Examiner has failed to establish a prima facie case of anticipation and the rejection is reversed.

2. Claim 40 stands rejected under 35 U.S.C. § 103 as obvious over Gunderson in view of Chandler.

Gunderson is discussed herein. The Examiner acknowledges that Gunderson does not teach application of the method to hCG. (Ans. 7.) Thus the Examiner relies on Chandler for teaching a method for the detection of a plurality of reactive sites on an analyte (see page 75, lines 20-30, where

Chandler teaches detection of hCG with antibodies to the alpha subunit). (Ans. 7.) The Examiner concludes that

> [i]t would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to detect HCG using the assay of Gunderson since Gunderson expressly teach detection of proteins using antibody detection methods (see paragraph 0663 and paragraph 0670, where Gunderson lists many different protein targets) and since Chandler teaches a very similar detection method to that of Gunderson, simply with the polyclonal antibodies being used as the label rather than attached to the solid support. An ordinary practitioner would have been motivated to substitute the assays of Gunderson for detection of HCG of Chandler in order to permit more complete capture of the HCG using the polyclonal antibodies, since polyclonal antibodies comprise sets of antibodies which detect many different epitopes of the target, permitting detection even where the epitope of the monoclonal antibody may be less available.

(Ans. 7-8.)

When determining whether a claim is obvious, an Examiner must make "a searching comparison of the claimed invention – *including all its limitations* – with the teaching of the prior art." *In re Ochiai*, 71 F.3d 1565, 1572 (Fed. Cir. 1995) (emphasis added). Thus, "obviousness requires a suggestion of all limitations in a claim." *CFMT, Inc. v. Yieldup Intern. Corp.*, 349 F.3d 1333, 1342 (Fed. Cir. 2003) (citing *In re Royka*, 490 F.2d 981, 985 (CCPA 1974)). Moreover, as the Supreme Court recently stated, "*there must be some articulated reasoning* with some rational underpinning to support the legal conclusion of obviousness." *KSR Int'l v. Teleflex Inc.*, 127 S. Ct. 1727, 1741 (2007) (quoting *In re Kahn*, 441 F.3d 977, 988 (Fed. Cir. 2006) (emphasis added)).

Appellants again argue that Gunderson does not teach or suggest allowing different reactants carried on a microsphere to react respectively with different reactive sites on an analyte to form reactive site pairs, wherein are distinguishable by their fluorescence intensity but not their fluorescence wavelength. (App. Br. 14.) Appellants further argue that Chandler teaches that a bead carries one type of reactant and that if the reactant has reacted with an analyte, the intensity of the measurement signal indicates that the reaction has taken place. (App. Br. 14.)

As discussed herein, we have found that the Examiner has failed to establish that Gunderson teaches different reactant sites on a single microsphere bead with detection of reactivity by fluorescence intensity but not fluorescence wavelength, as claimed. Nor does the Examiner rely on Chandler for this teaching.

The test of obviousness is "whether the teachings of the prior art, taken as a whole, would have made obvious the claimed invention." *In re Gorman*, 933 F.2d 982, 986 (Fed. Cir. 1991). For the reasons articulated with respect to the anticipation rejection over Gunderson, we do not find that the cited prior art teaches each of the claim limitations, and the rejection of claim 40 for obviousness is reversed.

3. Claims 7, 21, 35 and 45 stands rejected under 35 U.S.C. § 103 as obvious over Nolan in view of Gunderson.

The Examiner acknowledges that Gunderson does not teach the use of flow cytometric detection. (Ans. 8.) Thus the Examiner relies

on Nolan for teaching the detection of a plurality of reactive sites on an analyte comprising:

> (1) providing a population of microspheres …, wherein each of the microspheres carries a plurality of reactants capable of reacting respectively with the plurality of reactive sites (see figures 1 and 5 and column 4, lines 45-67, where each microsphere carries a nucleic acid that can react with a target nucleic acid), (2) forming reactant-reactive site pairs which are allowing the reactants and the reactive sites to react (see column 4, lines 45-67, example 1, where target nucleic acid is mixed with primers bound to microspheres), thereby detectably distinguishable from each other (see column 4, example 1, where ddNTPs are added making the primer detectable) and Nolan teaches a nucleic acid analyte, where the reactive site is an allelic locus and the reactant is a fluorescently labeled probe (see column 4, example 1, where the probe is extended with a fluorescent ddNTP so that the probe is specific for one allele and is fluorescently labeled); and (3) detecting the reactant-reactive site pairs formed, whereby the presence or absence of each of the plurality of reactive sites on the analyte is determined (see column 5, lines 10-17, where the primer beads are analyzed by flow cytometry to detect the presence of reactive sites).

(Ans. 8-9.)

The Examiner concludes that

> [i]t would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Gunderson to detect using flow cytometry or OLA [oligonucleotide ligation assay] since Nolan states "multiplexed sequence determination is an important element of the new flow cytometry method.

(Ans. 9.)

Appellants contend that Nolan does not teach or suggest allowing different reactants carried on a microsphere to react respectively with different reactive sites on an analyte to form reactant-reactive site pairs, which are distinguishable by their fluorescence intensity but not their fluorescence wavelength. (App. Br. 9.) In response to Appellants' argument, the Examiner argues that it is Gunderson that is relied on for the disclosure of the use of "multiple probes on single beads." (Ans. 19.)

As discussed herein, we have found that the Examiner has failed to establish that Gunderson teaches different reactant sites on a single microsphere bead with detection of reactivity by fluorescence intensity but not fluorescence wavelength, as claimed. Nor does Nolan overcome this deficiency.

The Examiner argues Nolan discloses multiplexed sequence determination using flow cytometry. (Ans. 19-20.) The Examiner further argues that while Gunderson teaches that spatial definition is one approach to the analysis of probes, Gunderson also teaches that multiple assays can be performed where only one of the two alleles will result in a signal. (Ans. 20.) From this, the Examiner concludes that one of ordinary skill in the art could analyze the microspheres of Gunderson in the flow cytometric method of Nolan (App. Br. 21.) The Examiner further concludes that "an ordinary practitioner would have been motivated to multiplex the detection in order to detect multiple different oligonucleotide hybridizations using fewer beads an in a more multiplexed, faster detection method. (Ans. 20.)

Appellants argue that "Nolan teaches repeating a process for different bases that are fluorescently labeled to determine a nucleotide base identity,"

and that "Nolan does not teach or suggest allowing different reactants carried on a microsphere to react respectively with different reactive sites on an analyte to form reactant-reactive site pairs, which are distinguishable by their fluorescence intensity but not their fluorescence wavelength." (App. Br. 9.)

The test of obviousness is "whether the teachings of the prior art, taken as a whole, would have made obvious the claimed invention." *In re Gorman*, 933 F.2d 982, 986 (Fed. Cir. 1991). We find no evidence before us that one of ordinary skill in the art would have been able to detect multiple reactant microspheres, with a reactant-reactive site pair distinguishable by fluorescence intensity but not by their fluorescence wavelength.

In view of the above the rejection of the claims for obviousness is reversed.

4. Claim 16 stands rejected under 35 U.S.C. § 103 as obvious over Iannone in view of Gunderson.

As acknowledged by the Examiner, Gunderson does not teach the use multiple organisms, as claimed. (Ans. 10.) Iannone is relied on for disclosing a method for the detection of a plurality of reactive sites on an analyte (see abstract, where Iannone teaches the method can detect SNPs), the method comprising:

> (1) providing a population of microspheres (see page 132; column 1, subheading "microspheres"), wherein each of the microspheres carries a plurality of reactants capable of reacting respectively with the plurality of reactive sites (see page 133, column 1 and page 134, figure I),

> (2) forming reactant-reactive site pairs which are allowing the reactants and the reactive sites to react (see page 133, where capture probes interact with Oligonucleotide ligation products), thereby detectably distinguishable from each other (see figure 1) and Iannone teaches a nucleic acid analyte, where the reactive site is an allelic locus and the reactant is a fluorescently labeled probe (see figure I),
> (3) detecting the reactant-reactive site pairs formed, whereby the presence or absence of each of the plurality of reactive sites on the analyte is determined. (see figure 1 and page 133, column 2).

(Ans. 11.)

"Iannone teaches the use of multiple different patients in a multiplex assay (see page 139, column 2)." (Ans. 11.)

The Examiner concludes that

> [i]t would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Gunderson to detect multiple patients as taught by Iannone since an ordinary practitioner would have been motivated to multiplex the detection in order to detect multiple different oligonucleotide hybridizations using fewer beads and in a more multiplexed, faster detection method.

(Ans. 11.)

Appellants argue that because claim 16 depends from claim 14, that Appellants' arguments with respect to the anticipation rejection over Gunderson, as well as the obviousness rejection over Gunderson in view of Nolan or Iannone and King are repeated. (App. Br. 13.) In addition Appellants argue that the cited references do not teach or suggest allowing different reactants carried on a microsphere to react respectively with different reactive sites on an analyst to form reactant-reactive site pairs,

which are distinguishable by their fluorescence intensity but not their fluorescence wavelength. (App. Br. 13.)

As discussed herein, we have found that the Examiner has failed to establish that Gunderson teaches different reactant sites on a single microsphere bead with detection of reactivity by fluorescence intensity but not fluorescence wavelength, as claimed. Ionnone fails to make up for this deficiency. The rejection of claim 16 for obviousness is reversed.

5. Claim 15 stands rejected under 35 U.S.C. § 103 as obvious over Gunderson in view of Nolan or Iannone and in further view of King.

The Examiner admits that Gunderson in combination with either Nolan or Iannone do not teach application of the method to BRCA1. (Ans. 11-12.) The Examiner relies on King which discloses that detection of BRCA1 is significant for detection of inherited breast cancer (see column 1). (Ans. 12.)

The Examiner concludes that

> [i]t would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to detect BRCAl using the method of Gunderson … in view of either Nolan … or Iannone since King expressly teaches that detection of BRCAl is important for both diagnostic and therapeutic applications (see column 2, lines 29-52) and since the method of Gunderson … in view of either Nolan … or Iannone is capable of detecting which allele is present in a patient and in normal or abnormal breast tissue.

(Ans. 12.)

Appellants argue that because claim 15 depends from claim 14, that Appellants' arguments with respect to the anticipation rejection over Gunderson are repeated. (App. Br. 12.) In addition Appellants argue that Iannone does not teach or suggest allowing different reactants carried on a microsphere to react respectively with different reactive sites on an analyte to form reactant-reactive site pairs, which are distinguishable by their fluorescence intensity but not their fluorescence wavelength. (App. Br. 11.)

As discussed herein, we have found that the Examiner has failed to establish that Gunderson teaches different reactant sites on a single microsphere bead with detection of reactivity by fluorescence intensity but not fluorescence wavelength, as claimed. We find that neither, Nolan, Ionnone or King make up for this deficiency. In view of the above, the rejection of claim 15 for obviousness is reversed.

SUMMARY

The anticipation and obviousness rejections are reversed.

No time period for taking any subsequent action in connection with this appeal may be extended under 37 C.F.R. § 1.136(a).

REVERSED

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