

UNITED STATES PATENT AND TRADEMARK OFFICE

**BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES**

Ex parte
MARK CHANDLER and MICHAEL SPAIN

Appeal 2007-3120
Application 09/791,894
Technology Center 1600

DECIDED: May 30, 2008

Before TONI R. SCHEINER, DEMETRA J. MILLS, and ERIC GRIMES,
Administrative Patent Judges.

SCHEINER, *Administrative Patent Judge.*

DECISION ON APPEAL

This is an appeal under 35 U.S.C. § 134 from the final rejection of claims 1-15. The claims stand rejected as unpatentable over the prior art. We have jurisdiction under 35 U.S.C. § 6(b).

We reverse.

STATEMENT OF THE CASE

“Diagnostic and genetic analysis can be subject to measurement errors” (Spec. 3). “[F]actors leading to measurement error include: instrument calibration error, samples that include substances which interfere with the assay, reagent (including sample) omission, and the hook effect” (*id.*). “[T]he present invention relates to methods of . . . internally calibrating multiplexed assays” (*id.*), and “provides internal standards which can be used to at least partially compensate for measurement errors in multiplexed assays” (*id.*).

Claims 1-15 stand rejected under 35 U.S.C. § 103(a) as unpatentable over Lehn (U.S. Patent 5,567,627, issued October 22, 1996), in view of Walt (U.S. Patent 6,327,410 B1, issued December 4, 2001).

Claims 1-4 are representative, and read as follows:

1. A method of internally calibrating a multiplexed assay comprising:
 - (a) exposing a mixture comprising a pooled population of at least one subset of particles to a reagent mixture comprising at least one type of signal ligand, wherein the particles within a subset are: (i) similarly sized; (ii) exhibit at least one characteristic fluorescent classification parameter that distinguish the particles of one subset from those of another; and (iii) comprise a tracer or a ligand binding partner specific to a target ligand, the ligand binding partner associated with one subset of particles may be the same as or different from the ligand binding partner associated with another subset of particles, and the target ligand is chosen from analytes suspected of being in a sample, tracers, and the at least one type of signal ligand, provided either that: (i) a specific target ligand is an analyte chosen from interfering factors and characteristic sample components excluding the analyte or analytes of interest, or (ii) the particles of at least one subset comprise a known concentration of a specific ligand binding partner corresponding to an analyte of interest, the concentration being chosen to test for a hook effect, or (iii) in one or more groups of subsets of particles, the particles in the subsets of the group comprise the same tracer or a ligand binding partner

corresponding to the same target ligand but at known concentrations that vary with each member of the one or more selected groups;

(b) passing the exposed particles through an examination zone; and
(c) collecting data as the exposed particles pass through the examination zone relating to: (i) one or more characteristic classification parameters of each particle including data on signal intensities, and at least either (ii) the presence or absence of a complex formed between the ligand binding partner and target ligand or (iii) the apparent concentrations of the one or more particular analytes of interest associated with each member of the one or more selected groups, or both (ii) and (iii).

2. A method according to claim 1, further comprising substantially simultaneously with collecting, classifying each particle according to its subset and, when the target ligand is an analyte, quantifying an amount of analyte associated with each subset.

3. A method according to claim 1, wherein the at least one subset of particles is at least two subsets of particles, the particles of a first subset comprise ligand binding partner specific for target ligand chosen from analytes and the particles of at least a second subset comprise ligand binding partner specific for target ligand chosen from signal ligand.

4. A method according to claim 3, wherein the at least one subset of particles is at least five subsets of particles, the particles of the second, third, fourth, and fifth subset of particles comprise ligand binding partner specific for the same signal ligand but at known concentrations that vary with each subset.

FINDINGS OF FACT (FF)

Terms Used in the Present Specification and Claims

1. “Multiplexed assay” means “an assay that can detect and/or measure multiple targets” (Spec. 3: 11-12).
2. “The term ‘particle’ refers to a microsphere or bead . . . for use in flow cytometric multiplexed assays” (Spec. 7: 7-8), and “[t]he term ‘subset of particles’ refers to a group of particles sharing essentially the same

characteristic classification parameters . . . [such that] they can be identified as belonging to the same group of particles and also distinguished from the particles of another group" (Spec. 7: 9-12), "for example by . . . fluorescent signature" (Spec. 18: 4).

3. "Calibrating" means "compensating for measurement error and/or detecting sources of measurement error" (Spec. 3: 10-11), and "internally calibrating" means "the use of internal standards and controls for calibrating assays" (Spec. 3: 18-19).

4. "[F]actors leading to measurement error include: instrument calibration error, samples that include substances which interfere with the assay, reagent (including sample) omission, and the hook effect" (Spec. 3: 4-6).

5. "'[I]nternal,' when used in conjunction with standard or control, e.g., 'internal standard' or 'internal control,' . . . mean[s] capable of being included in an assay reagent mixture or in any sample" (Spec. 3: 15-17).

6. "Target" means "any substance desired to be analyzed, including analytes, internal standards, internal controls, or any component of the assay reagent mixture" (Spec. 3: 12-14).

7. "'[S]ignal ligand' refers to a ligand which is capable of being detected. A signal ligand can be, for example, any substance having associated therewith a detectable label such as a fluorescently- or radioactively-tagged antibody or antigen" (Spec. 8: 19-21).

8. "'[T]arget ligand' refers to a ligand in (or put into) the reagent mixture or sample, which is capable of coupling with a ligand binding partner" (Spec. 9: 2-3).

9. “‘[T]racer’ refers to a signal ligand that competes with a target ligand for binding to a particular ligand binding partner. For example, if a fluorescently-labeled antigen ‘A’ and its unlabeled antigen ‘A’ counterpart both compete for binding to a particular antibody associated with a particle, the fluorescently-labeled antigen is a ‘tracer,’ the unlabeled antigen is a ‘target ligand,’ and the antibody is a ‘ligand binding partner.’” (Spec. 9: 7-11).

10. “‘[I]nterfering factor’ relates to any substance in the sample or reagent mixture which can interfere with the coupling of a ligand binding partner and a target ligand leading to an artificially low result” (Spec. 9: 11-13).

11. “Analyte” means “any substance suspected of being present in a sample” (Spec. 3: 14-15), and “‘analyte of interest’ refers to the analyte or analytes desired to be analyzed” (Spec. 8: 18).

Lehnen

12. Lehnen describes “simultaneous assays to detect multiple analytes of interest in a sample using a mixture of multiple discrete populations of complementary binding moieties attached to a solid support” (Lehnen, col. 2, ll. 54-56), e.g., “a microsphere, that may be detected by flow cytometry techniques” (Lehnen, col. 4, ll. 3-4).

13. Lehnen does not describe fluorescent microspheres. “[I]dentification of the analyte does not depend upon the size or other physical characteristic of the solid support to which the complementary binding moiety is bound” (Lehnen, col. 4, ll. 4-7), and “[a]t least four, generally five or six analytes

may be assayed simultaneously using a single bead size - and/or a single fluorochrome" (Lehnen, col. 4, ll. 11-17).

14. In Lehnen's method, a population of microspheres of the same size is divided into subpopulations (three, for example), and each subpopulation is associated with a binding moiety specific for one of three analytes (A, B, and C) of interest. The three subpopulations are combined in "unique nonadditive proportions" (1:2:7, for example), and incubated with a sample and a fluorescent labeling reagent. After unbound labeling reagent is removed, a predetermined number of microspheres is counted by flow cytometry, and the relative intensity of the labeling agent associated with each microsphere is collected and the data plotted as a histogram of number of events versus relative label intensity. If none of the three analytes is present in the sample, only background fluorescence will be detected. If B is the only analyte present, then 20% of the microspheres counted will be fluorescently labeled, if A and B are present, then 30% of the microspheres counted will be fluorescently labeled, and so on. Where two or three analytes are present in differing amounts, separately identifiable subpopulations will be observed based on the relative intensity of label detected. (Lehnen, col. 5, l. 24 to col. 6, l. 8.)

15. Lehnen teaches that a simplified flow cytometer can be used in his method "since there is no need to discriminate based upon bead size . . . [and] only fluorescence need be measured" (Lehnen, col. 10, ll. 1-6), that is, "[t]he occurrence of a fluorescent event of intensity at or above a preselected intensity is recorded" (Lehnen, col. 10, ll. 23-24).

16. “As an internal control, included in the reagent mix can be a subpopulation of microspheres coated with the first of a binding pair wherein the second member of the binding pair is ubiquitous in samples to be analyzed . . . Thus the absence of a positive resultant indicates that the method was employed erroneously, thus serving as a control on the reproducibility of the method when, for example large numbers of samples are analyzed” (Lehn, col. 10, ll. 29-36).

Walt

17. Walt describes a method combining “a bead-based analytic chemistry system in which beads, also termed microspheres, carrying different chemical functionalities may be mixed together while the ability is retained to identify the functionality of each bead using an optically interrogatable encoding scheme (an ‘optical signature’)” and an array or “substrate comprising a patterned surface containing individual sites that can bind or associate individual beads” (Walt, col. 4, ll. 36-43).

18. “[B]ioactive agents may be synthesized on the beads, and then the beads are randomly distributed on a patterned surface” (Walt, col. 4, ll. 46-48).

19. “Since the beads are first coded with an optical signature, . . . the array can later be ‘decoded’, i.e., after the array is made, a correlation of the location of an individual site on the array with the bead or bioactive agent at that particular site can be made” (Walt, col. 4, ll. 48-52).

20. Thus, “each subpopulation of microspheres comprise[s] a unique optical signature or optical tag that can be used to identify the unique bioactive agent of the subpopulation of microspheres” (Walt, col. 13, ll. 10-

13), and “[t]he bioactive agent is designed so that in the presence of the analyte(s) to which it is targeted, an optical signature of the microsphere . . . is changed” (Walt, col. 7, ll. 46-49).

DISCUSSION

The Examiner rejected claims 1-15¹ under 35 U.S.C. § 103(a) as unpatentable over Lehnен in view of Walt.

Claim 1, which represents the invention in its broadest aspect, is directed to a method of internally calibrating a multiplexed assay, where “a pooled population of at least one subset of particles” is exposed to a reagent mixture in order to compensate for, or detect sources of measurement error. Claim 1 covers a multitude of embodiments, including an embodiment where a subset population of microspheres is associated with a ligand binding partner specific for a “characteristic sample component[],” other than the analyte of interest. In this embodiment, the presence of a complex formed between the particles and the characteristic (i.e., ubiquitous) sample component confirms that a sample is actually present in the reaction mixture, while its absence is an indication that the sample was inadvertently omitted from the reaction mixture.

As an initial matter, we note that Appellants contend that neither Lehnен nor Walt “teach[es] or suggest[s] any method for internally calibrating a multiplexed assay” (App. Br. 10, 11), while the Examiner contends that “the preamble does not breath[e] life and meaning into the

¹ The Examiner’s omission of claim 9 in the initial statement of the rejection (Ans. 3) appears to be a typographical error because claim 9 is addressed in the body of the rejection in addition to claims 1-8 and 10-15. Appellants recognized that the rejection applies to all of claims 1-15 (App. Br. 5).

claim . . . [and] there is no mention of calibrating the assay anywhere else in the claim” (Ans. 8).

Both Appellants’ and the Examiner’s arguments fall wide of the mark because Lehnен explicitly teaches that “a subpopulation of microspheres coated with the first of a binding pair wherein the second member of the binding pair is ubiquitous in samples to be analyzed” can be included in the reagent mix, “thus serving as a control on the reproducibility of the method when . . . large numbers of samples are analyzed” (Lehnен, col. 10, ll. 29-36; FF 16). Clearly, Lehnен describes a method for internally calibrating a multiplexed assay that is consistent with Appellants’ definition of “internally calibrating” (FF 3, 4, 5).

The residual issue raised by this appeal, then, is whether the prior art teaches or suggests using a subset of particles that exhibits “at least one characteristic fluorescent classification parameter that distinguish[es] the particles of one subset from those of another” in Lehnен’s method. That is, does the prior art teach or suggest using intrinsically fluorescent particles in Lehnен’s method?

The Examiner acknowledges that “Lehnен does not teach the step of using a fluorescent classification parameter to distinguish particles of one subset from another” (Ans. 4). However, the Examiner notes that Walt “teach[es] the use of beads with . . . [a fluorescent] optical signature” (Ans. 4), which “provide[s] a good signal-to-noise ratio for decoding” (*id.*), and also teaches that “unique tags may be generated . . . by covalently attaching . . . [fluorescent] dyes to the surface of the beads, or . . . by entrapping the dye within the bead” (*id.*), and “varying both the composition of the mixture

(i.e., the ratio of one dye to another) and the concentration of the dye (leading to differences in signal intensity)” (*id.*).

The Examiner contends that “it would have been obvious to use a fluorescent classification parameter to distinguish subsets of particles, as taught by Walt et al, in the method of Lehnен, in order to provide a good signal-to-noise ratio for decoding” (Ans. 4-5).

Appellants contend that Lehnен “teaches the desirability of *not* discriminating [between beads] based upon physical characteristics or any other characteristics of the beads” (App. Br. 14-15), in order to “allow[] the methods to be used with a relatively low cost flow cytometer” (App. Br. 15).

In our view, Appellants have the better argument.

[T]he Examiner bears the burden of establishing a *prima facie* case of obviousness based upon the prior art. “[The Examiner] can satisfy this burden only by showing some objective teaching in the prior art or that knowledge generally available to one of ordinary skill in the art would lead that individual to combine the relevant teachings of the references.”

In re Fritch, 972 F.2d 1260, 1265 (Fed. Cir. 1992) (citations omitted, bracketed material in original). “[T]here 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)).

We agree with Appellants that the Examiner has not established a *prima facie* case of obviousness based on the proposed combination of references. Walt’s method depends on retaining the ability to identify the exact makeup of individual beads, after they are randomly arrayed, using an optically interrogatable encoding scheme (FF 17). The reason given by the

Examiner that one of ordinary skill would have used Walt's optically tagged fluorescent microspheres in Lehnens' method is that individual fluorescent signatures would provide a good signal-to-noise ratio for fluorescently decoding Lehnens' microparticles. However, decoding (even by bead size, or unique fluorescent label) is what Lehnens' method explicitly avoids (FF 13, 14, 15).

In this case, we find that the Examiner has not established that one of ordinary skill in the art would have had a reason to substitute Walt's beads, with their unique optical signatures, for Lehnens' non-fluorescent, one-size-fits-all beads. That is, we find that the Examiner has not established that one of ordinary skill in the art would have had a reason to modify Lehnens' method by replacing Lehnens' non-fluorescent particles with particles exhibiting "at least one characteristic fluorescent classification parameter that distinguish[es] the particles of one subset from those of another," as required by the claims.

Accordingly, the rejection of claims 1-15 as unpatentable over the combined teachings of Lehnens and Walt is reversed.

REVERSED

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DAFFER MCDANIEL LLP
P.O.BOX 684908
AUSTIN, TX 78768