

**UNITED STATES PATENT AND TRADEMARK OFFICE**

---

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

---

Ex parte ALONSO CASTRO

---

Appeal No. 2003-0892  
Application No. 09/454,385<sup>1</sup>

---

ON BRIEF

---

Before SCHEINER, ADAMS and GREEN, 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 1-12, all of the claims in the application.

Claim 1, the sole independent claim, is representative:

1. A method for identifying single molecules having a target DNA or RNA sequence comprising the steps of:
  - selecting a primer having a 3'-hydroxyl group at one end and having a sequence of nucleotides to specifically hybridize with an identifying sequence of nucleotides in the target DNA;
  - hybridizing the primer to the identifying nucleotide sequences of the target DNA or RNA sequence;
  - extending the primer along the target sequence by progressively binding a plurality of nucleotides to the primer that are complementary to the corresponding nucleotides on the target sequence to form a reporter molecule, where the complementary nucleotides include nucleotides labeled with a fluorophore; and
  - detecting fluorescence emitted by fluorophores on individual reporter molecules by a process selected from the group consisting of flow cytometry and single molecule

---

<sup>1</sup> Application for patent filed December 3, 1999. According to appellant, this application claims the benefit of provisional application serial no. 60/113,139, filed December 18, 1998.

electrophoresis to identify the target DNA or RNA sequence.

The references relied on by the examiner are:

Conrad	5,652,099	Jul. 29, 1997
Mandecki	5,736,332	Apr. 7, 1998
Albrecht et al. (Albrecht)	6,265,163	Jul. 24, 2001

Castro & Shera (Castro), "Single-Molecule Electrophoresis," Analytical Chemistry, Vol. 67, No. 18, pp. 3181-3186 (September 15, 1995)

Claims 1, 2, 5 and 6 stand rejected under 35 U.S.C. § 102(b) as anticipated by Mandecki; claims 1-3 and 5-7 stand rejected under 35 U.S.C. § 102(e) as anticipated by Albrecht; and claims 1-12 stand rejected under 35 U.S.C. § 103(a) as unpatentable over Conrad and Castro.

We reverse these rejections.

### BACKGROUND

The rapid and efficient detection of specific nucleic acid sequences in biological samples plays a central role in a variety of fields . . . One of the most commonly used techniques for the detection of specific nucleic acid sequences is the Southern blot[,]. . . in which the fragments to be interrogated have been size-separated by gel electrophoresis and transferred from the gel to a nylon nitrocellulose filter. A radioactive probe is then added to the filter so that hybridization takes place. After washing away the excess probe, the band containing the target nucleic acid is detected by exposing an X-ray film to the filter.

Despite its popularity, Southern blotting suffers from some limitations: it involves a series of manually intensive procedures that cannot be run unattended and cannot be readily automated . . .

The use of automated probes brings up . . . safety and environmental concerns. The lack of adequate sensitivity is another limitation, which has been partially addressed by the development of the polymerase chain reaction (PCR) and related target amplification methods . . . Amplification methods, however, may introduce ambiguities resulting from contamination or from variability in amplification efficiency.

Specification, pages 1-2.

The present invention provides a "non-radioactive approach for the ultrasensitive detection of specific sequences" which "combines the advantages of flow-based

analytical systems (system automation, speed, reproducibility) with the unsurpassed sensitivity of single-molecule detection” (specification, page 4). “A primer having a 3'-hydroxyl group at one end and having a sequence of nucleotides sufficiently homologous to hybridize with an identifying sequence of nucleotides in [a] target DNA or RNA is selected . . . [and then] hybridized to the identifying sequence of nucleotides[.] [A] reporter molecule is synthesized on the target sequence by extending the primer by progressively binding nucleotides to the primer that are complementary to the corresponding nucleotides of the DNA or RNA [target] sequence, where the complementary nucleotides include nucleotides labeled with a fluorophore. Fluorescence emitted by fluorophores on individual reporter molecules is detected to identify the target DNA or RNA sequence” (id., pages 2-3). According to appellant, “[t]he sensitivity of this method allows for the direct detection of specific genes without the need for using amplification methods” (id., page 4).

## DISCUSSION

### Anticipation by Mandecky or Albrecht

“[E]very 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, 43 USPQ2d 1030, 1032 (Fed. Cir. 1997). Moreover, “the Patent Office has the initial burden of coming forward with some sort of evidence tending to disprove novelty.” In re Wilder, 429 F.2d 447, 450, 166 USPQ2d 545, 548 (CCPA 1970).

Mandecky describes solid phase particles coated with multiple copies of an oligonucleotide probe, wherein the sequence of the probe is encoded on the memory element of a transponder physically associated with the particle (Examples 1 and 3).

“[T]arget DNA of unknown sequence is labeled with a fluorophore and combined with transponder particles carrying known oligonucleotides . . . [t]he transponders are analyzed to detect the fluorescence or color originating from a label that indicates that target DNA has bound to the probe attached to the . . . transponder, and the information stored electronically in the transponder is decoded” (column 1, lines 49-68). As appellant points out, “each transponder has a surface that is covered with the encoded oligonucleotides . . . so that a large fluorescent signal is obtained from the multitude of labeled target sequences rather than extended reporter molecules on each target sequence” (Brief, page 3). As the examiner puts it, Mandeck “detect[s] the fluorescence emitted by each and every fluorophore of each and every individual reporter molecule . . . at the same time and joins them together” (Answer, page 7).

Albrecht describes a method for identifying and isolating differentially expressed genes or polymorphic genes. “[D]ifferently labeled populations of DNAs from cell or tissue sources whose gene expression is to be compared [are provided and] . . . competitively hybridized with reference DNA cloned on solid phase supports” (column 2, lines 28-48). The reference oligonucleotides “are synthesized on the surface of a solid phase support, such as a microscopic bead or a specific location on an array of synthesis locations on a single support, such that populations of identical . . . sequences are produced in specific regions. That is, the surface of each support, in the case of a bead, or of each region, in the case of an array, is derivatized by copies of only one type of tag complement having a particular sequence” (column 10, lines 18-29). Appellant argues that Albrecht thus “fail[s] to teach [appellant’s] recited method steps for identifying single target molecules or for extending a primer molecule along the single target sequences so that individual target sequences can be identified

readily” (Brief, pages 5-6). The examiner does not disagree.

Thus, the teachings of the Mandeck and Albrecht do not appear to be in dispute. Rather the dispute is one of claim construction. According to the examiner, “the claim is open to . . . detection of every fluorophore and every reporter simultaneously” (Answer, page 8), and “detection of multiple different molecules along with the single molecule” (*id.*, page 7) is permitted. We disagree.

The claimed method requires “identifying single molecules having a given target DNA or RNA sequence” (claim 1) by selecting a primer complementary to the target sequence; hybridizing the primer to the target sequence; forming a fluorescent reporter molecule by extending the primer along the target sequence, in a manner complementary to the target sequence, by progressively binding a plurality of nucleotides, some of which are fluorophore-labeled, to the primer; and detecting the fluorescent target/reporter molecule by flow cytometry or single molecule electrophoresis. While it may be, as the examiner argues, that there is “no requirement that the detection proceed without detecting additional molecules” (Answer, page 7), each target molecule must be detected as an individual entity - we see nothing in the claim which is open to detection of an aggregate of multiple target molecules.

Inasmuch as neither Mandeck nor Albrecht detects a signal from an individual, discrete reporter molecule, neither reference meets every limitation of the claim 1, the broadest claim on appeal. Accordingly, we find that neither reference anticipates the claims, and we reverse the rejection of the claims over Mandeck, as well as the rejection of the claims over Albrecht.

#### Obviousness

Conrad describes “fluorescent structural analogs of the non-fluorescent

nucleosides commonly found in DNA and RNA, methods of their derivatization and subsequent use in the synthesis of fluorescent oligonucleotides . . . having prescribed sequences” (column 1, lines 13-19). According to the examiner (Answer, pages 5-6),

Conrad teaches a method for identifying target DNA or RNA sequences (column 27, lines 50-55) comprising: a) selecting a primer of more than 15 nucleotides . . . having a 3' hydroxyl group at one end and having a sequence of nucleotides to specifically hybridize with an identifying sequence of nucleotides in the target DNA (column 27, lines 55-60), b) hybridizing the primer to the identifying nucleotide sequences of the target RNA sequence (column 27, lines 60-65), c) extending the primer along the target sequence by progressively binding a plurality of nucleotides to the primer that are complementary to the corresponding nucleotides on the target sequence to form a reporter molecule, where the complementary nucleotides include rUTP nucleotides labeled with a fluorophore and unlabelled rATP, rCTP, and rGTP nucleotides . . . [and] d) detecting fluorescence emitted by fluorophores on individual reporter molecules to identify the target DNA sequence (column 28, lines 2-10).

Castro describes “single-molecule electrophoresis,” which “promises to combine the advantages of free-solution capillary electrophoresis (system automation, speed, reproducibility) with the unsurpassed sensitivity of single-molecule detection” (page 3186). According to the examiner, “[i]t would have been prima facie obvious to one having ordinary skill in the art at the time the invention was made to combine the method of Conrad with the use of single molecule electrophoresis as taught by Castro[,]” “for the express advantages of improved speed, reproducibility and unsurpassed sensitivity” (Answer, page 6).

Appellant argues that one skilled in the art would not have had reason to detect Conrad’s fluorescent polynucleotides using Castro’s method because “[t]he advantages of single molecule electrophoresis taught by Castro [ ] are for sorting and detecting single molecules by size . . . but [sic, not?] for detecting the presence or absence of a fluorescent probe” (Brief, pages 6-7). We disagree. Castro specifically teaches that the “technique is sensitive enough to detect and analyze a small, single-fluorophore

molecule, as well as fluorescently labeled DNA” (page 3184), opening the way “to develop fluorescence immunoassay, hybridization, and DNA fingerprinting techniques that do not require extensive DNA amplification using the polymerase chain reaction [ ] or other methods” (page 3186). Nor are we persuaded that the present invention excludes the use of Conrad’s “inherently fluorescent nucleosides” in the present fluorophore-labeled target/reporter molecules, as appellant argues (Brief, page 5).

Nevertheless, the examiner’s description of Conrad’s teachings notwithstanding, appellant asserts that Conrad does not “extend[ ] a primer sequence along a target molecule after the primer has first been hybridized to a target sequence to form an extended hybridized reporter molecule with incorporated [n]ucleotides labeled with a fluorophore” (Brief, page 6), and thus, “does not teach a process for forming a reporter molecule directly on a target molecule” (*id.*), as specifically required by the claims on appeal. The examiner has not responded to appellant’s assertion in any way. Moreover, having reviewed Conrad, especially those portions particularly relied on by the examiner, we are hard pressed to find anything that corresponds to the examiner’s description, and it appears to us that appellant’s assertion is well founded.

The initial burden of presenting a prima facie case of obviousness rests on the examiner. In re Oetiker, 977 F.2d 1443, 1445, 24 USPQ2d 1443, 1444 (Fed. Cir. 1992). Moreover, findings of fact underlying an obviousness rejection, as well as conclusions of law, must be made in accordance with the Administrative Procedure Act, 5 U.S.C. 706 (A),(E) (1994), see Zurko v. Dickinson, 527 U.S. 150, 158, 119 S.Ct. 1816, 1821, 50 USPQ2d 1930, 1934 (1999), and must be supported by substantial evidence within the record. See In re Gartside, 203 F.3d 1305, 1315, 53 USPQ2d

1769, 1775 (Fed. Cir. 2000). On this record, we find an insufficient factual basis to support the examiner's conclusion, and we reverse the rejection of claims 1-12 under 35 U.S.C. § 103 because the examiner has not established that all of limitations of the claims on appeal were taught or would have been suggested by the prior art.

REVERSED

	)	
Toni R. Scheiner	)	
Administrative Patent Judge	)	
	)	
	)	
	)	BOARD OF PATENT
	)	
Donald E. Adams	)	APPEALS AND
Administrative Patent Judge	)	
	)	INTERFERENCES
	)	
	)	
	)	
Lora M. Green	)	
Administrative Patent Judge	)	

Appeal No. 2003-0892  
Application No. 09/454,385

Page 9

Ray G. Wilson  
LC/BPL MS D412  
Los Alamos National Laboratory  
Los Alamos, NM 87545