

The opinion in support of the decision being entered today was not written for publication and is not binding precedent of the Board.

Paper No. 17

UNITED STATES PATENT AND TRADEMARK OFFICE

---

BEFORE THE BOARD OF PATENT APPEALS  
AND INTERFERENCES

---

Ex parte JOSEPH R. LAKOWICZ

---

Appeal No. 1999-2814  
Application 08/990,539

---

ON BRIEF

---

Before WILLIAM F. SMITH, SCHEINER, and GRIMES, Administrative Patent Judges.

GRIMES, Administrative Patent Judge.

DECISION ON APPEAL

This is a decision on appeal under 35 U.S.C. § 134 from the examiner's final rejection of claims 1, 2, 10, 14, 16, 21, and 23-33, all of the claims remaining. Claims 1 and 27 are representative and read as follows:

1. A method for determining a base sequence of a nucleotide strand comprising the steps of:
  - (a) providing a first probe, comprising a fluorescent metal-ligand complex coupled to a first oligonucleotide having a sequence complementary to a first fragment of said strand;

- (b) adding said first probe to a sample that contains said first fragment to form a first mixture containing a first reaction product of said first probe and said first fragment;
- (c) exposing said first mixture to an exciting amount of radiation;
- (d) detecting fluorescence of said first metal-ligand complex;
- (e) identifying a first base sequence of said first fragment based on fluorescence of said first metal-ligand complex;
- (f) providing a second probe, comprising a second fluorescent metal-ligand complex coupled to a second oligonucleotide having a sequence complimentary to a second fragment of said strand differing from said first fragment by at least one base;
- (g) adding said second probe to a sample that contains said second fragment to form a second mixture containing a second reaction product of said second probe and said second fragment;
- (h) exposing said second mixture to an exciting amount of radiation;
- (i) detecting fluorescence of said second metal-ligand complex;
- (j) identifying a second base sequence of said second fragment based on fluorescence of said second metal-ligand complex;
- (k) comparing said second base sequence with said first base sequence to identify a difference between the first and second sequences and thereby determine a base sequence of said nucleotide strand;

wherein the metal in each said fluorescent metal-ligand complex is selected from the group consisting of Co, Cr, Cu, Mo, Rh, W, Re, Os, Ir, and Pt;

wherein said detection utilizes measurement of fluorescence lifetime; and

wherein autofluorescence is suppressed by fluorescence gating.

27. In a method for determining the nucleotide base sequence of a nucleic acid molecule, which comprises the steps of:
- a) annealing said nucleic acid molecule with a primer molecule able to hybridize to said nucleic acid molecule;
  - b) incubating the annealed mixture with four different deoxynucleoside triphosphates, a polymerase, and a nucleic acid synthesis terminating agent which terminates synthesis at a specific nucleotide base; and
  - c) separating the products of step b);

the improvement comprising detecting the separated products via a fluorescent metal-ligand complex, wherein when the metal in each said fluorescent metal-ligand complex is Ru, the complex is selected from the group consisting of [Ru(2,2'-bipyridyl)<sub>2</sub>(1,10-phenanthroline-9-isothiocyanate)]<sup>2+</sup>, [Ru(4,7-diphenyl-1,10-phenanthroline)<sub>2</sub>(4,4'-dicarboxylic acid-2,2'-bipyridine)]<sup>2+</sup>, [Ru(4,7-diphenyl-1,10-phenanthroline)<sub>2</sub>(4-methyl, 4'-carboxylic acid-2,2'-bipyridine)]<sup>2+</sup>, [Ru(4,7-diphenyl-1,10-phenanthroline(SO<sub>3</sub>Na)<sub>2</sub>)<sub>2</sub>(4,4'-dicarboxylic acid-2,2'-bipyridine)]<sup>2+</sup>, [Ru(4,7-diphenyl-1,10-phenanthroline(SO<sub>3</sub>Na)<sub>2</sub>)<sub>2</sub>(4-methyl,4'-carboxylic acid-2,2'-bipyridine)]<sup>2+</sup>, and [Ru bis(2,2'-bipyridyl) (phenanthroline-maleamide)].

The examiner relies on the following references:

Bannwarth, "Bathophenanthroline-Ru(II) complexes as nonradioactive labels for dideoxy DNA sequencing," Analytical Biochemistry, Vol. 181, pp. 216-219 (1989)

Zhang et al. (Zhang), "Use of non-cross-linked polyacrylamide for four-color DNA sequencing by capillary electrophoresis separation of fragments up to 640 bases in length in two hours," Analytical Chemistry, Vol. 67, pp. 4589-4593 (1995)

Soper et al. (Soper), "On-Line Fluorescence Lifetime Determinations in Capillary Electrophoresis," Analytical Chemistry, Vol. 67, pp.4358-4365 (1995)

Terpetschnig et al. (Terpetschnig), "Fluorescence polarization immunoassay of a high-molecular-weight antigen using a long wavelength absorbing and laser diode-excitable metal-ligand complex," Analytical Biochemistry, Vol. 240, pp. 54-59 (1996)

Claims 1, 2, 10, 14, 16, 21, and 23-33 stand rejected under 35 U.S.C. § 103 as obvious over Zhang, Bannwarth, Terpetschnig, and Soper.

We reverse.

#### Background

The specification discloses the use of fluorescent metal-ligand complexes as labels in DNA sequencing processes. The specification discusses a DNA sequencing method based on hybridization of different probes to the sample DNA. See, e.g., pages 6-7. In this method, a “first probe” is made, which consists of a “fluorescent metal-ligand complex . . . coupled to a first oligonucleotide having a sequence complementary to [a] first fragment.” Id., page 6. This probe is mixed with a “first fragment” of the sample DNA and exposed to radiation to excite the fluorescent metal-ligand complex. The fluorescence is then detected, thereby identifying the base sequence of the first fragment. Id.

Next, a “second probe” is made, which consists of a “fluorescent metal-ligand complex . . . coupled to a second oligonucleotide having a sequence complementary to [a] second fragment,” where the sequence of the second fragment “differ[s] from the first fragment by at least one base.” Id. The second probe is then mixed with a “second fragment” of the sample DNA, exposed to radiation to excite the fluorescent metal-ligand complex, and the fluorescence is detected, thereby identifying the base sequence of the second fragment. Id., pages 6-7. The base sequence of the sample DNA is then determined by comparing the different sequences of the first and second fragments. “The steps

of the invention may be repeated to sequentially identify further bases of the nucleotide strand, until the strand is completely sequenced.” Id., page 19.

The specification also discloses that fluorescent metal-ligand complexes can be used as fluorescent labels in a dideoxy sequencing process. See pages 19-20 (Examples 1 and 2).

### Discussion

The claims are directed to both the sequencing-by-hybridization method (claims 1, 2, 10, 14, 16, 21, and 23-26) and an improvement in a standard dideoxy sequencing method, the improvement being the use of a fluorescent metal-ligand complex as a label (claims 27-33). Even though the claims are directed to two distinctly different inventions, the examiner applied the same obviousness rejection, based on the same set of references, to all the claims.

In essence, the examiner relied on Zhang as teaching a DNA sequencing method, and relied on Bannwarth and Terpetschnig as teaching use of fluorescent metal-ligand complexes in similar methods. See the Examiner’s Answer, pages 4-5.<sup>1</sup> The examiner concluded that

[i]t would have been prima facie obvious to one having ordinary skill in the art at the time the invention was made . . . to substitute the labels of Bannwarth into the method of Zhang since Bannwarth [sic] expressly notes that these labels are very sensitive, very stable and may be ideal in some sequencing procedures. Bannwarth . . . further motivates the synthesis of analog complexes. This statement motivates the use of the alternative osmium metal ligand complexes as taught by Terpetschnig since Terpetschnig states

---

<sup>1</sup> The examiner cited Soper as “teach[ing] the use of fluorescence lifetime determinations in DNA sequencing ladders in capillary gel electrophoresis.” Examiner’s Answer, page 5. Soper was apparently cited to meet the limitation in claim 1 that the “detection utilizes measurement of fluorescence lifetime.” However, as discussed *infra*, we conclude that this limitation is not critical to the obviousness analysis. Therefore, we will not further discuss Soper.

“The osmium complex described in this report has the favorable property of a long absorption wavelen[g]th and high anisotropy.”

Id., page 6.

Appellant argues that the examiner has not made out a prima facie case. Appellant argues that Terpetschnig cannot properly be combined with the other references because it is directed to labeling of antibodies and antigens for use in an immunoassay, not labeling of DNA for DNA sequencing as in the claims. Appeal Brief, pages 6-7. Appellant also argues that the prior art provides no motivation to use the label disclosed by Terpetschnig in DNA sequencing methods. Id., pages 8-9. Finally, Appellant argues that none of the cited references suggest the invention of claims 1, 2, 10, 14, 16, 21, and 23-26, i.e., the multiple-probe, sequencing-by-hybridization method. Id., pages 9-10.

“In rejecting claims under 35 U.S.C. § 103, the examiner bears the initial burden of presenting a prima facie case of obviousness. Only if that burden is met, does the burden of coming forward with evidence or argument shift to the applicant.” In re Rijckaert, 9 F.3d 1531, 1532, 28 USPQ2d 1955, 1956 (Fed. Cir. 1993). “In determining whether obviousness is established by combining the teachings of the prior art, the test is what the combined teachings of the references would have suggested to those of ordinary skill in the art.” In re GPAC Inc., 57 F.3d 1573, 1581, 35 USPQ2d 1116, 1123 (1995) (internal quotations omitted).

After reviewing the record, we agree with Appellant that the prior art does not support a prima facie case of obviousness. Of the references cited by the

examiner, only Terpetschnig discloses a fluorescent metal-ligand complex meeting the limitations of the instant claims.<sup>2</sup> Thus, a prima facie case of obviousness for any of the claims would require a person of ordinary skill in the art to combine the osmium-containing fluorescent metal-ligand complex taught by Terpetschnig with a DNA sequencing method such as that taught by Zhang or Bannwarth.

We do not agree with Appellant's argument that Terpetschnig is nonanalogous art. "Two criteria have evolved for determining whether prior art is analogous: (1) whether the art is from the same field of endeavor, regardless of the problem addressed, and (2) if the reference is not within the field of the inventor's endeavor, whether the reference still is reasonably pertinent to the particular problem with which the inventor is involved." In re Clay, 966 F.2d 656, 658, 23 USPQ2d 1058, 1060 (Fed. Cir. 1992). The examiner argues that the relevant field of endeavor is "clinical chemistry," an assertion for which no evidentiary support has been offered and with which Appellant disagrees.

We find it unnecessary to resolve this dispute, however, since Terpetschnig clearly satisfies the second criterion for analogous art because it is "reasonably pertinent to the particular problem" of fluorescent labels in DNA sequencing. The pertinence of Terpetschnig is evidenced by Bannwarth, who discloses the use of a fluorescent metal-ligand complex as a label in DNA sequencing. In view of Bannwarth's disclosure of ruthenium-containing

---

<sup>2</sup> The claims either exclude ruthenium-containing complexes entirely (claims 1, 2, 10, 14, 16, 21, 23-26, and 32) or encompass only specific ruthenium-containing complexes not including the bathophenanthroline complexes of Bannwarth (claims 27-31 and 33).

complexes for labeling in DNA sequencing, those skilled in the art would have recognized the pertinence of Terpetschnig's osmium-containing fluorescent metal-ligand complexes for the same purpose. We therefore agree with the examiner that Terpetschnig is analogous art.

Even though the cited references are analogous, however, "[t]here must be some reason, suggestion, or motivation found in the prior art whereby a person of ordinary skill in the field of the invention would make the combination." In re Oetiker, 977 F.2d 1443, 1447, 24 USPQ2d 1443, 1446 (Fed. Cir. 1992). An adequate showing of motivation to combine requires "evidence that 'a skilled artisan, confronted with the same problems as the inventor and with no knowledge of the claimed invention, would select the elements from the cited prior art references for combination in the manner claimed.'" Ecolochem, Inc. v. Southern Calif. Edison Co., 227 F.3d 1361, 1375, 56 USPQ2d 1065, 1075 (Fed. Cir. 2000) (quoting In re Rouffet, 149 F.3d 1350, 1357, 47 USPQ2d 1453, 1456 (Fed. Cir. 1998)).

The examiner relies on Bannwarth's statement that

these [bathophenanthroline Ru(II)] complexes can be measured in a time-resolved mode after excitation by laser pulses with very high sensitivity and due to the fact that they are very stable they seem to be ideal nonisotopic labels in those sequencing procedures which operate with just one single fluorescent primer. Nevertheless it should be possible to synthesize analog complexes showing different emission spectra and use them in those sequencing procedures where four different dyes are necessary.

Bannwarth, page 218, left-hand column.

The examiner argues that

[a]n ordinary practitioner would have been motivated to substitute the labels of Bannwarth into the method of Zhang since Bannwarth [sic] expressly notes that these labels are very sensitive, very stable and may be ideal in some sequencing procedures. Bannwarth . . . in the above quotation further motivates the synthesis of analog complexes. This statement motivates the use of the alternative osmium metal ligand complexes as taught by Terpetschnig since Terpetschnig states “The osmium complex described in this report has the favorable property of a long absorption wavelen[g]th and high anisotropy. . . . An ordinary practitioner would have been motivated to substitute the Osmium label of Terpetschnig into the DNA sequencing method of Zhang in view of Bannwarth . . . for the advantage of a long absorption wavelength and high anisotropy.

Examiner’s Answer, pages 6-7.

We agree with Appellants that the cited references would not have suggested the methods of the instant claims to a person of ordinary skill in the art. The examiner argues that a skilled artisan would have been motivated to combine Bannwarth’s fluorescent metal-ligand label with Zhang’s sequencing method based on Bannwarth’s statement that the disclosed labels “seem to be ideal nonisotopic labels in those sequencing procedures which operate with just one single fluorescent primer.” Zhang’s sequencing method, however, does not operate with just one single fluorescent primer. The method described by Zhang employs four primers, each labeled with a different fluorescent label. See the abstract (“Four-color DNA cycle sequencing was performed.” (emphasis added)). The examiner provides no explanation of why a person of ordinary skill in the art would combine a fluorescent label that is disclosed to be ideal for one type of DNA sequencing process with a different DNA sequencing process.

Second, the examiner relies on Bannwarth's statement that "it should be possible to synthesize analog complexes showing different emission spectra and use them in those sequencing procedures where four different dyes are necessary," as evidence that those skilled in the art would have been motivated to use Terpetschnig's osmium-containing complex as a label for DNA sequencing. The examiner, however, cites no evidence showing that those skilled in the art would have considered Terpetschnig's osmium-containing complex to be an "analog" of Bannwarth's ruthenium-containing complex. The fluorescent metal-ligand complexes of the two references contain different metals and different ligands complexed to the metal. Compare the structure shown in Bannwarth's Figure 1 with that of Terpetschnig's Scheme 1. The examiner has cited no evidence supporting his position that those of skill in the art would consider the two compounds to be "analogous."

Nor has he provided evidence that Terpetschnig's fluorescent metal-ligand complex has an emission spectrum that would lead those skilled in the art to combine it with Bannwarth's fluorescent metal-ligand complex. There is no evidence of record, for example, that the two fluorescent labels emit light of different wavelengths. Terpetschnig compares the emission spectra of an osmium-containing complex and a ruthenium-containing complex (see Figure 2), but the ruthenium-containing complex shown is different from that of Bannwarth. The record does not indicate the emission spectrum of Bannwarth's ruthenium-containing complex.

Finally, the examiner points to Terpetschnig's disclosure that the osmium-containing complex "has the favorable property of a long absorption wavelength and a high anisotropy." In view of this teaching, the examiner argues, "[a]n ordinary practitioner would have been motivated to substitute the Osmium label of Terpetschnig into the DNA sequencing method of Zhang in view of Bannwarth . . . for the advantage of a long absorption wavelength and high anisotropy." This argument is also unpersuasive. Terpetschnig discloses an osmium-containing fluorescent dye for use in immunoassays. While a long absorption wavelength and high anisotropy are evidently desirable properties in that context, the record contains no evidence that they are also desirable properties in a fluorescent label for use in DNA sequencing. Thus, the evidence does not support the examiner's reliance on this statement to provide motivation to combine the cited references.

We conclude that the examiner's rejection is not supported by an adequate "reason, suggestion, or motivation" to combine the cited references. "Combining prior art references without evidence of such a suggestion, teaching, or motivation simply takes the inventor's disclosure as a blueprint for piecing together the prior art to defeat patentability—the essence of hindsight." In re Dembiczak, 175 F.3d 994, 999, 50 USPQ2d 1614, 1617 (Fed. Cir. 1999) (citations omitted).

With respect to the sequencing-by-hybridization claims, Appellant argues that "[a]ll of the sequencing references [relied on by the examiner] show the use of a single primer. In contrast, [claims 1, 2, 10, 14, 16, 21, and 23-26] require the use of two different oligonucleotides. . . . Clearly, that feature is absent from all

references and consequently is not rendered obvious by the combination.”

Appeal Brief, pages 9-10.

We agree. The examiner argues that both Bannwarth and Zhang teach this claim limitation, because “Bannwarth expressly shows the use of two different primers on page 217, figure 1. . . . Separately, Zhang teaches the use of primers in which each primer has a different label.” Examiner’s Answer, page 11. This argument is unpersuasive. Bannwarth’s Figure 1 indeed shows three different primers, but the accompanying text makes clear that the primers are shown merely to illustrate the synthesis of the final, ruthenium-labeled primer. See page 217, right-hand column (citation omitted):

Primer **1** represents a 24 mer universal primer for M13 (18). In primer **2**, this universal primer was extended at the 5’-end by 5’-amino-5’-deoxythymidine in order to generate specifically a primary 5’-amino group. A specific covalent coupling of the Ru (bathophenanthroline) complex to this group via an amide bond yielded primer **3**.

The examiner has provided no explanation of how this disclosure would have suggested a sequencing method such as that of instant claim 1, i.e., one in which the sequence of a target DNA is determined based on the hybridization of two or more probes which differ in sequence.

Zhang also fails to suggest this aspect of the claimed method. The examiner argues that “Zhang teaches the use of primers in which each primer has a different label,” Examiner’s Answer, page 11, but points to nothing in Zhang that teaches or suggests primers or probes which differ in sequence, as required by the instant claims. Therefore, the rejection of claims 1, 2, 10, 14, 16,

21, and 23-26 also fails because the examiner has not shown that the cited references suggest all of the limitations of the claimed process.

Summary

We reverse the rejection under 35 U.S.C. § 103 because the cited references do not support a prima facie case of obviousness.

REVERSED

William F. Smith	)	
Administrative Patent Judge	)	
	)	
	)	
	)	BOARD OF PATENT
Toni R. Scheiner	)	
Administrative Patent Judge	)	APPEALS AND
	)	
	)	INTERFERENCES
	)	
Eric Grimes	)	
Administrative Patent Judge	)	

EG/dym

Appeal No. 1999-2814  
Application No. 08/990,539

Rothwell Figg Ernst and Kurz  
Suite 701 E  
555 13<sup>th</sup> Street NW  
Washington DC 20004