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BEFORE THE PATENT TRIAL AND APPEAL BOARD

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*Ex parte* MICHAEL R. KNAPP, JILL M. BAKER, ANDREA W. CHOW, ANNE R. KOPF-SILL, and MICHAEL SPAID

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Appeal 2017-011016
Application 14/252,755[1]
Technology Center 1600

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Before JEFFREY N. FREDMAN, TIMOTHY G. MAJORS, and RACHEL H. TOWNSEND, *Administrative Patent Judges*.

TOWNSEND, *Administrative Patent Judge*.

DECISION ON APPEAL

This is an appeal under 35 U.S.C. § 134(a) involving claims to methods of detecting a nucleic acid of interest, which have been rejected as obvious. We have jurisdiction under 35 U.S.C. § 6(b).

We reverse.

STATEMENT OF THE CASE

"One of the most powerful and basic technologies for nucleic acid detection is nucleic acid amplification." (Spec. ¶ 4.) Using the polymerase chain reaction ("PCR") one can amplify a nucleic acid of interest prior to its

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[1] Appellants identify the real party in interest as Caliper Life Sciences, Inc., which is a PerkinElmer Company. (Appeal Br. 3.)

detection, which is helpful "because it is easier to detect many copies of a nucleic acid than it is to detect a single copy." (*Id.*) "[A] number of high throughput approaches to performing PCR and other amplification reactions have been developed, e.g., involving amplification reactions in microfluidic devices, as well as methods for detecting and analyzing amplified nucleic acids in or on the devices." (*Id.* at ¶ 6.) However, "certain technical difficulties persist in amplifying and detecting nucleic acids, particularly rare copy nucleic acids. This is particularly true where the amplification reagents amplify a high copy nucleic acid in a given sample in addition to the rare nucleic acid and the two nucleic acids differ by only one or a few nucleotides in the same sample." (*Id.* at ¶ 7.) The invention is directed to a high throughput method of single molecule amplification. (*Id.* at ¶ 11.)

Claims 1, 3–9, and 11–17 are on appeal. Claim 1 is representative and reads as follows:

> 1. A method of detecting a nucleic acid of interest, the method comprising:
>
> aliquotting a sample comprising the nucleic acid of interest and one or more additional nucleic acids into a plurality of reaction mixtures, wherein at least one of the plurality of reaction mixtures is a single copy reaction mixture comprising a single copy of the nucleic acid of interest, wherein the plurality of reaction mixtures additionally comprise at least one additional reaction mixture comprising at least one copy of the additional nucleic acid;
>
> simultaneously subjecting the plurality of reaction mixtures in parallel to one or more amplification reactions while flowing each of the plurality of reaction mixtures through one of a plurality of channels of a microfluidic device; and,
>
> detecting the nucleic acid of interest in the at least one single copy reaction mixture;

> wherein at least one of the plurality of reaction mixtures is formulated in an aqueous phase of an emulsion comprising aqueous droplets suspended in and surrounded by an immiscible liquid.

(Appeal Br. 17.)

The following ground of rejection by the Examiner is before us on review:

Claims 1, 3–9, and 11–17 under 35 U.S.C. § 103 as unpatentable over Curcio,[2] Vogelstein,[3] Lagally,[4] Ghadessy,[5] Nisisako,[6] Dower,[7] Tawfik,[8] Katsura,[9] Fouillet,[10] and Obeid.[11]

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[2] Curcio et al., *Continuous Segmented-Flow Polymerase Chain Reaction for High-Throughput Miniaturized DNA Amplification*, 75(1) Anal. Chem., 1–7, 2003.
[3] Vogelstein et al., US 6,440,706, issued Aug. 27, 2002.
[4] Lagally et al., *Single-molecule DNA amplification and analysis in an integrated microfluidic device*, 73(3) Anal. Chem., 565–70, 2001.
[5] Ghadessy et al., *Directed evolution of polymerase function by compartmentalized self-replication*, 98(a) PNAS, 4552–4557, 2001.
[6] Nisisako et al., *Formation of Droplets Using Branch Channels in a Microfluidic Circuit*, SICE 2002, 957–959, 2002.
[7] Dower et al., *In vitro selection as a powerful tool for the applied evolution of proteins and peptides*, 6(3) Curr. Opin. Chem. Biol., 390–398, 2002.
[8] Tawfik et al., *Man-made cell-like compartments for molecular evolution*, 16(7) Nat. Biotechnol., 652–656, 1998.
[9] Katsura et al., *Indirect micromanipulation of single molecules in water-in-oil emulsion*, 22(2) Electrophoresis, 289–293, 2001.
[10] Fouillet et al., US 2001/0041357 A1, published Nov. 15, 2001.
[11] Obeid et al., *Microfabricated Device for DNA and RNA Amplification by Continuous-Flow Polymerase Chain Reaction and Reverse Transcription-Polymerase Chain Reaction with Cycle Number Selection*, 75(2) Anal. Chem., 288–295, 2003.

## DISCUSSION

The Examiner finds that Curcio teaches a method of detecting a nucleic acid of interest in a sample using amplification in which the sample is aliquotted into a plurality of reaction mixtures in a channel of a microfluidic device, each reaction mixture separated from one another by immiscible filler fluid, resulting in segmented flow. (Final Action 4–5; Advisory Action 2.) The Examiner acknowledges that Curcio does not expressly teach the reaction mixture is a single copy reaction mixture. (Final Action 7.) And, the Examiner also acknowledges that Curcio's segmented flow does not involve the reaction mixture being formulated in an aqueous phase of an emulsion or as an aqueous droplet therein. (Advisory Action 2.)

The Examiner contends that "Lagally provides motivation and a reasonable expectation of success to apply single-molecule dilutions, used in PCR, to integrated microfluidic devices like Curcio's, which uses sub-microliter sample volumes." (Final Action 7.) The Examiner points to the following statements in Lagally as the motivation and reasonable expectation of success:

> "[t]he capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices" (pg. 566, col. 2)
>
> "[a]n integrated microdevice utilizing sub-microliter sample volumes with sensitivity to the single-molecule limit would avoid [amplification time and sensitivity] shortcomings and could serve as a platform for high-throughput parallelization" (pg. 566, col. 1)
>
> "[t]he detection of single DNA molecules will also facilitate single-cell and single-molecule studies to expose the genetic variation underlying ensemble sequence and expression averages" (Abs.).

(Final Action 7–8; *see also* Advisory Action 2.)

The Examiner contends that "a skilled artisan of ordinary creativity at the time of the invention would have been motivated to apply the single-molecule dilution principle of VOGELSTEIN to . . . Curcio, in view of the motivation to apply single-molecule PCR on microfluidic devices from Lagally, in order to 'accurately and quantitatively detect[] [single-copy or rare] genetic sequences in mixed populations of sequences.'" (Final Action 10–11.) According to the Examiner "VOGELSTEIN teaches the benefits of single-molecule amplifications to detect low concentration targets among high background non-targets with ratio-based concentration-determination techniques, which benefits would have immediately been recognized by a skilled artisan of ordinary creativity as applicable to microfluidic techniques which yield high-throughput . . . ." (Ans. 6).

The Examiner recognizes that Vogelstein's compartments are wells, but asserts that it would have been obvious to one having ordinary skill in the art to apply the single-molecule dilution scheme in other single compartments, citing Ghadessy. (Final Action 10–12.) According to the Examiner, Ghadessy suggests that the wells of Vogelstein can be replaced with water-in-oil emulsion compartmentalization as an equivalent alternative to those wells, where the individual self-replication reaction takes place in the compartment and there can be between $10^8$ to $10^9$ such compartments per milliliter. (*Id.* at 11–12.)

The Examiner further contends that "[a]ll of Nisisaki, Dower, Tawfik and Katsura provide further evidence as to the motivation to apply emulsions to single-molecule PCR with a reasonable expectation of success." (*Id.* at 12.) According to the Examiner "Nisisako teaches that 'a PCR method

using micro droplets as DNA containers has recently been proposed' such as w/o emulsions, which 'is under intensive study' and 'will become increasingly important,' including as applied to 'microfabricated devices.'" (*Id.*) The Examiner finds "Dower teaches emulsion compartmentalization (via a 'DNA concentration [that] is selected to insert, on average, a single DNA molecule in each droplet') of single nucleic acids for PCR (Fig. 3a; citing Tawfik, ref. 44)." (*Id.*) The Examiner finds that "Katsura demonstrates that w/o emulsions can be formed to compartmentalize a single nucleic acid for PCR." (*Id.* at 13.)

Regarding the reaction mixture being formulated as an aqueous droplet in an emulsion therein, the Examiner contends that emulsion droplets "were known substitutes for well-based droplets" in amplification, citing Nisisako, Dower, Tawfik, and Katsura. (Ans. 5.)

Finally, regarding the claim requirement of parallel PCR analysis on a continuous-flow device, the Examiner contends that Curcio itself provides motivation for such a modification "[t]o increase the total sample capacity." (Final Action 14.) The Examiner contends that Curcio "explicitly suggests to combine smaller-droplet single-molecule amplification systems with familiar highly-parallelized microfludic systems." (Ans. 6.) The Examiner bases that contention on a finding that

> Curcio specifically suggests that "[t]he use of miniaturized fluidics on a chip-based format is particularly attractive["] (pg. 7, col. 1, para. 1), and "[t]o increase the total sample capacity, many flow channels could be operated in parallel" (pg. 7, col. 1, para. 2). Such suggestions are also repeated in the "Conclusion" section on page seven.

(Advisory Action 2.) The Examiner, citing to Fouillet and Obeid, notes that such processing was routinely applied to continuous-flow PCR devices at

the time of the invention. (Final Action 14.) Thus, the Examiner concludes, one of ordinary skill in the art would have had a reasonable expectation of success in carrying out parallel PCR analysis.

We disagree with the Examiner's conclusion that the claimed invention would have been obvious from the teachings of the various references cited. Our reviewing Court has "outlined two classes of situations where 'obvious to try' is erroneously equated with obviousness under § 103." *In re Kubin*, 561 F.3d 1351, 1359 (Fed. Cir. 2009.) One of those "impermissible 'obvious to try' situations occurs where 'what was "obvious to try" was to explore a new technology or general approach that seemed to be a promising field of experimentation, where the prior art gave only general guidance as to the particular form of the claimed invention or how to achieve it.'" *Id.*

Emulsion droplet containers were a known alternative that seemed promising for PCR at the time of the invention. (*See* Nisisako at 1264 (

> To exploit the resulting droplets as small reaction/analysis chambers, cooperation with a manipulation method will be required. As a further project in our laboratory, we are working on the manipulation of micro droplets inside liquid layer by electric fields.);

(Ghadeesy at 4552 ("We foresee many applications of this [compartmentalized self-replication] technology.").) We agree with Appellants that the Examiner has failed to provide any teaching that would reasonably provide a suggestion with an expectation of success of using emulsion droplets as reaction chambers for PCR in a continuous flow system.

As Appellants note, not one of the references suggests use of emulsion droplets in flow systems. (Reply Br. 4.) Appellants explain that Curcio and

Obeid (the Group A references) are directed to slug flow PCR. (Appeal Br. 13.)

In the Final Action, the Examiner was of the opinion that Curcio's slug flow was reasonably considered an emulsion system. (Final Action 5–6.) However, the Examiner conceded in the Advisory Action dated January 19, 2017 that Curcio's slug flow was not an emulsion system. (*See* Ans. 3–4.) Nevertheless, the Examiner maintained the position of a reasonable expectation of success of using emulsion droplets in continuous flow systems like Curcio on the basis of the position that (1) "emulsion droplets (Applicants' "Group C") were known substitutes for well-based droplets used in digital amplification (Applicants' "Group B")" (Ans. 5) and (2) "Curcio specifically suggests that '[t]he use of miniaturized fluidics on a chip-based format is particularly attractive (pg. 7, col. 1, para. 1), and '[t]o increase the total sample capacity, many flow channels could be operated in parallel' (pg. 7, col. 1, para. 2)" (*id.* at 6). However, as Appellants note, Curcio teaches a slug flow system and "merely states that the physical design of their [slug flow] system could be further optimized and that the use of miniaturized fluidics on a chip-based format is particularly attractive." (Appeal Br. 11–13; Reply Br. 4–5.) In the absence of a teaching or suggestion by Curcio of an emulsion system, or some other teaching or evidence that systems such as Curcio's would remain functional when in emulsion systems, we disagree with the Examiner's finding that Curcio provides a reasonable expectation of success that an emulsion be used in a continuous flow system, much less a single droplet compartment within an emulsion.

Appellants explain that Lagally and Vogelstein (the Group B references) disclose single drop amplification on microwell plates, and that Ghadessy, Nisisako, Dower, Tawfik, and Katsura (the Group C references) taken together simply disclose PCR methods using emulsion droplets as reaction compartments. (Appeal Br. 13–15; Reply Br. 4.) We agree. Lacking in the Examiner's obviousness rejection is evidence that one skilled in the art would have had a reason to combine the single drop amplification methods on microwell plates of Group B and the emulsion droplets of Group C, neither of which have anything to do with fluid flow systems, with the slug flow continuous PCR systems of Group A with a reasonable expectation of success where none of the references "disclose use of emulsions in continuous flow systems." (Reply Br. 4.) At most, the Examiner has simply cited references which suggest a promising field of experimentation which is not sufficient on the record before us to establish obviousness under 35 U.S.C. § 103.

Consequently, we do not sustain the Examiner's rejection of claims 1, 3–9, and 11–17 as being obvious over Curcio, Vogelstein, Lagally, Ghadessy, Nisisako, Dower, Tawfik, Katsura, Fouillet, and Obeid.

## SUMMARY

We reverse the rejection of claims 1, 3–9, and 11–17 under 35 U.S.C. § 103 as unpatentable over Curcio, Vogelstein, Lagally, Ghadessy, Nisisako, Dower, Tawfik, Katsura, Fouillet, and Obeid.

## REVERSED