



**10x Genomics, Inc.,
Petitioner
v.
Bruker Spatial Biology, Inc.,
Patent Owner**

**IPR2023-01298
U.S. Patent No. 11,377,689**

Petitioner's Demonstratives for Oral Hearing

March 12, 2025

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

1

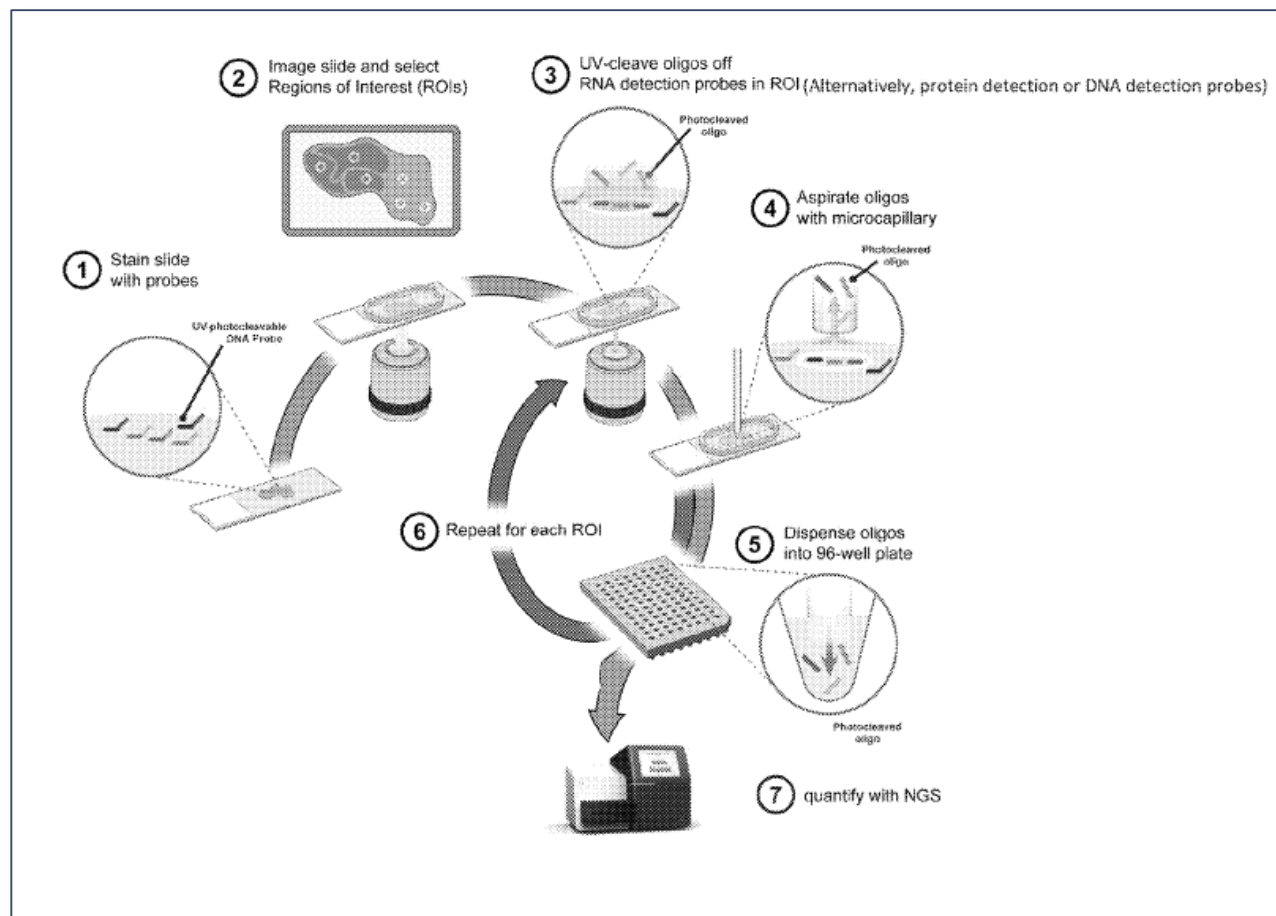
Overview

- The 689 patent (slides 3-13)
- Chell anticipates claims 1-30 (Ground 4) (slides 14-52)
- So-456 anticipates claims 1-8, 11-23, and 26-30 (Ground 1) (slides 53-85)
- So-456 renders claims 1-30 obvious (Ground 2) (slides 86-91)
- So-456 in view of Stahl further render claims 9-10 and 24-25 obvious (Ground 3) (slides 86-91)

The 689 Patent

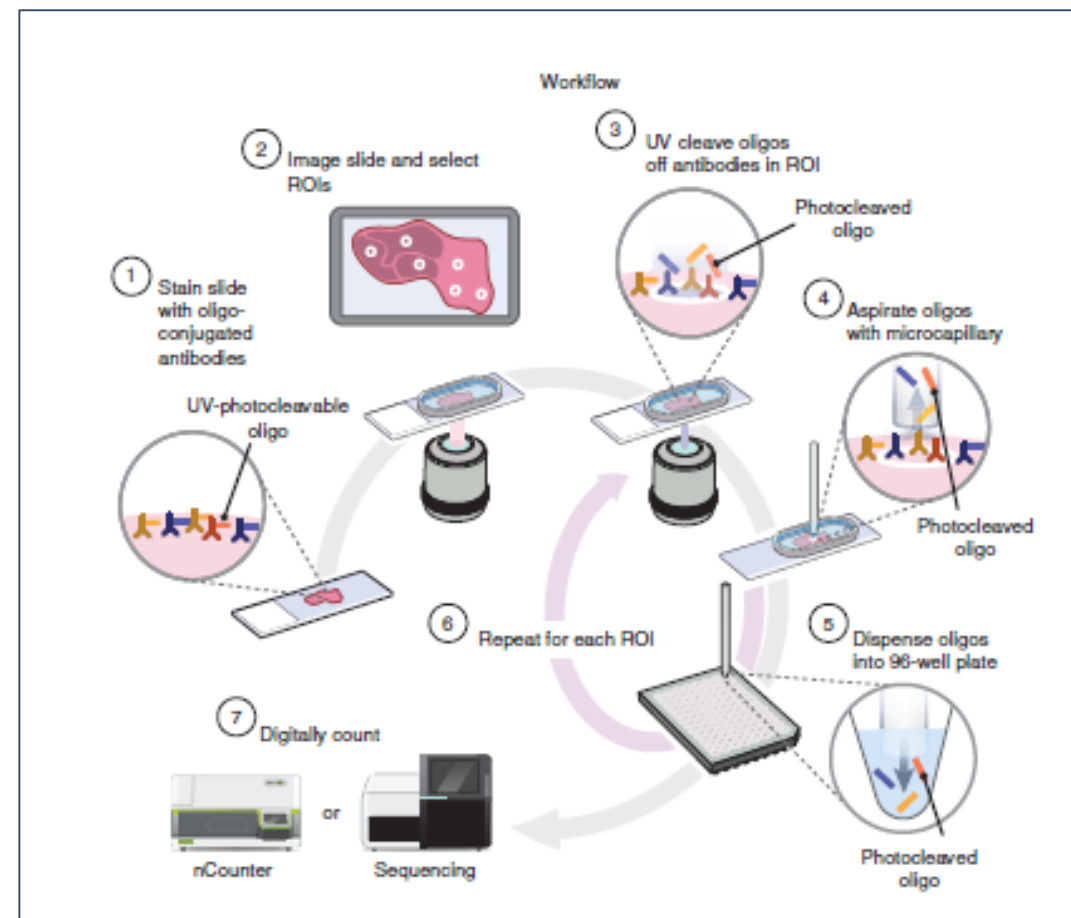
The 689 patent: drafted to describe Bruker's GeoMx

689 patent



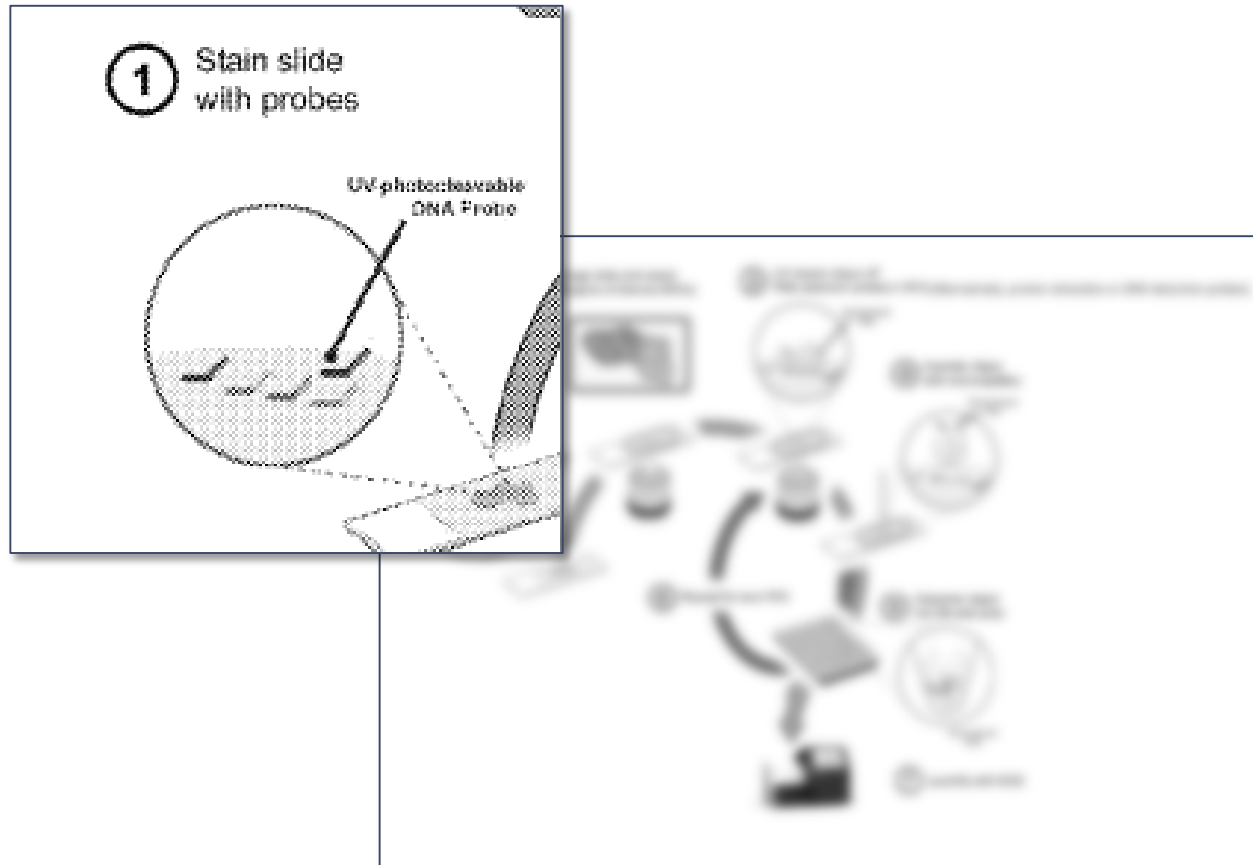
EX1001, Fig. 20

GeoMx

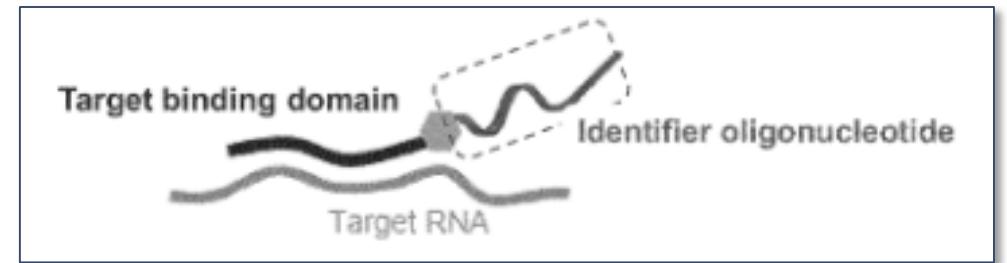


EX1013, Fig. 1A

(1) Add cleavable probes

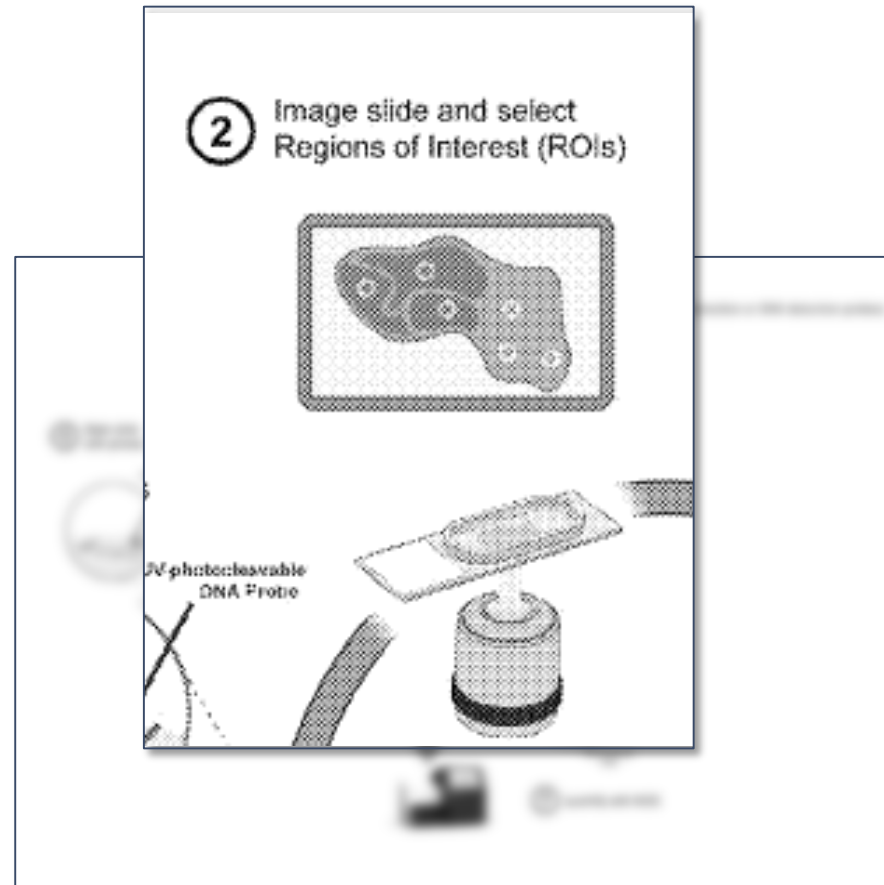


EX1001, Fig. 20



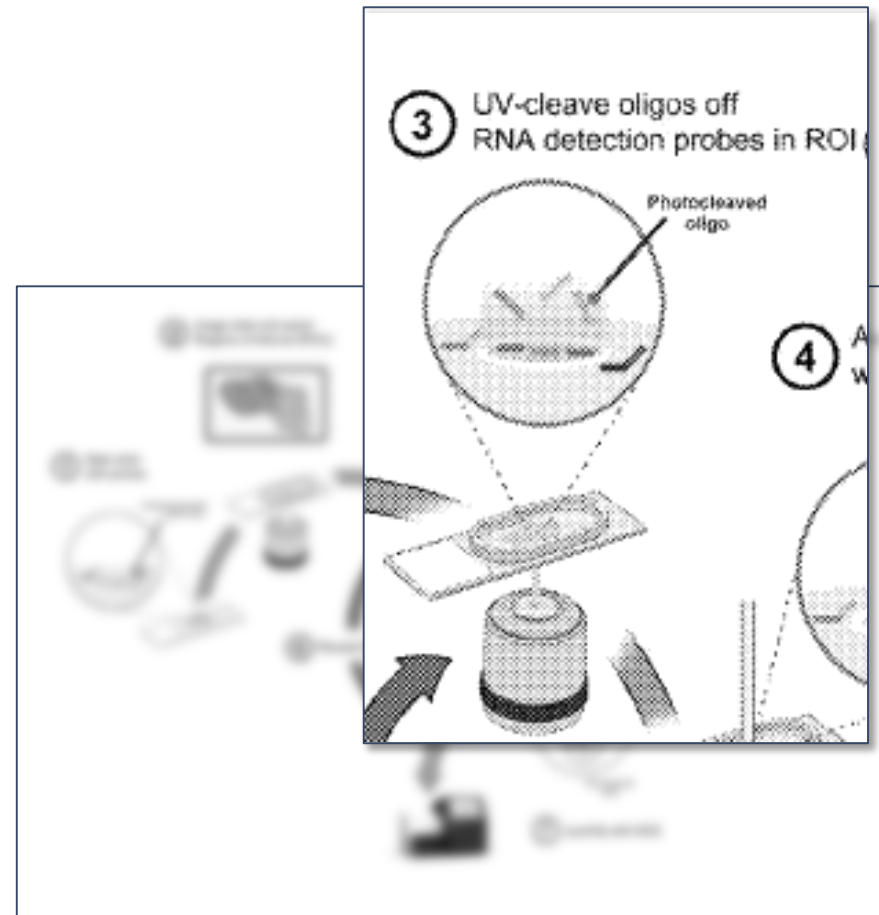
EX1001, Fig. 11

(2) Select “region of interest” (ROI)



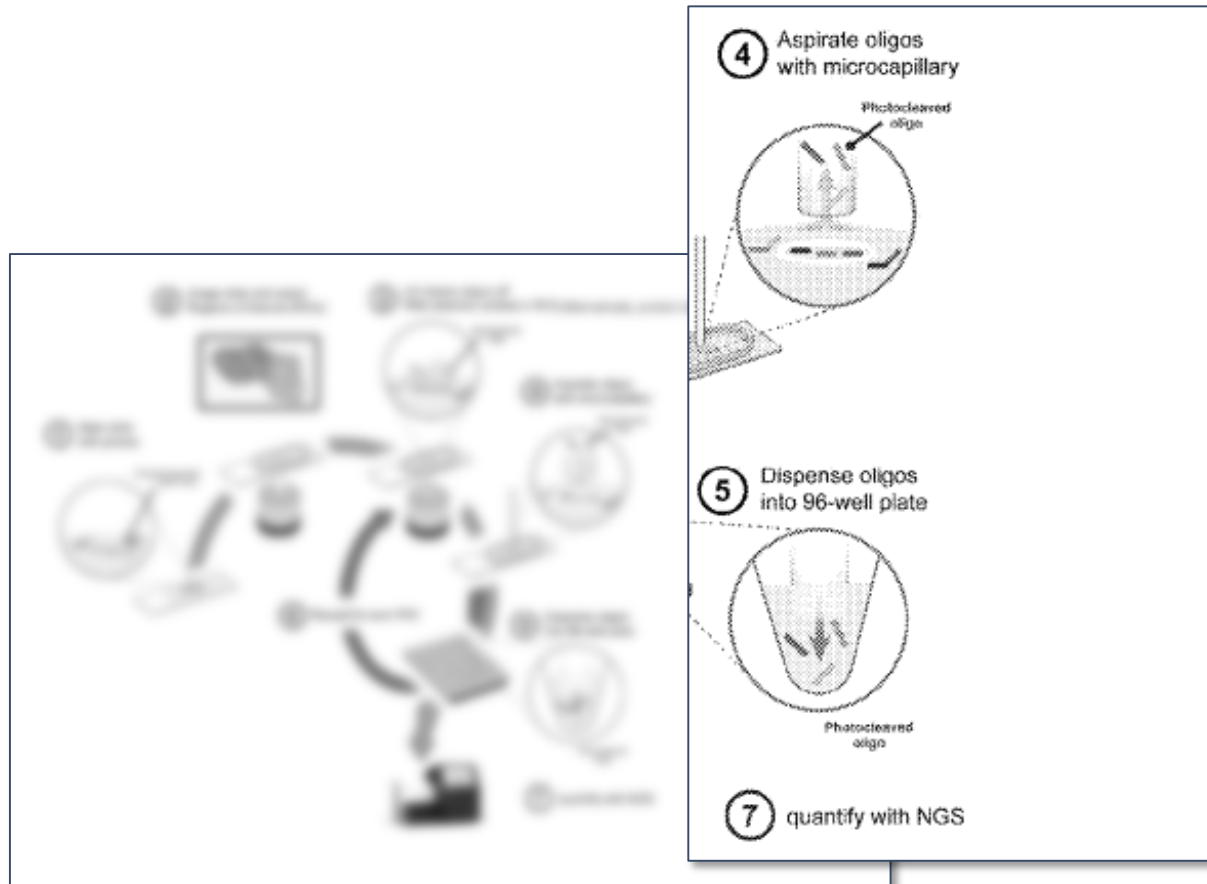
EX1001, Fig. 20

(3) Apply “force” to cleave off identifier oligonucleotide portion of probe



EX1001, Fig. 20

(4-7) Collect and analyze cleaved identifier oligonucleotides



EX1001, Fig. 20

The challenged claims – contacting step

Claim 1	Claim 16
1. A method for spatially detecting at least one target analyte in a first location and a second location of a tissue sample comprising:	16. A method for spatially detecting at least one target analyte in a first location and a second location of a tissue sample comprising:
a) contacting the tissue sample with a plurality of nucleic acid probes, wherein each of the nucleic acid probes comprise a target binding domain that binds to the at least one target analyte, wherein the tissue sample is treated to facilitate binding of the nucleic acid probes to the target analyte;	a) contacting the tissue sample with a first plurality of nucleic acid probes and a second plurality of nucleic acid probes, wherein each of the nucleic acid probes in the first plurality of nucleic acid probes comprise a target binding domain that binds to the at least one target analyte, wherein each of the nucleic acid probes in the second plurality of nucleic acid probes comprise a target binding domain that binds to the at least one target analyte, wherein the tissue sample is treated to facilitate binding of the first plurality of nucleic acid probes and the second plurality of nucleic acid probes to the at least one target analyte;

The challenged claims– ligating step (b) (claim 16 only)

Claim 1	Claim 16
	b) forming ligated probes by ligating each of nucleic acid probes from the first plurality of nucleic acid probes and each of nucleic acid probes from the second plurality of nucleic acid probes that are bound to each of identical molecules from the at least one target analyte ;

The challenged claims – collecting steps

Claim 1	Claim 16
b) collecting the nucleic acid probes, or portions thereof , bound to the at least one target analyte in a first location of the tissue sample under conditions that release the nucleic acid probes, or portions thereof, from the first location of the tissue sample;	c) collecting the ligated probes, or portions thereof , bound to each of the identical molecules from the at least one target analyte in a first location of the tissue sample under conditions that release the ligated probes, or portions thereof, from the first location of the tissue sample;
c) collecting the nucleic acid probes, or portions thereof , bound to the at least one target analyte in a second location of the tissue sample under conditions that release the nucleic acid probes, or portions thereof, from the second location of the tissue sample;	d) collecting the ligated probes, or portions thereof , bound to each of the identical molecules from the at least one target analyte in a second location of the tissue sample under conditions that release the probes, or portions thereof, from the second location of the tissue sample;

The challenged claims – extension steps

Claim 1	Claim 16
d) performing an extension reaction that incorporates at least one nucleic acid sequence that identifies the first location of the tissue sample into each of the nucleic acid probes, or portions thereof, collected in step (b), thereby forming a first plurality of extension products that comprise the nucleic acid probes, or portions thereof, collected in step (b) and the at least one nucleic acid sequence that identifies the first location of the tissue sample;	e) performing an extension reaction that incorporates at least one nucleic acid sequence that identifies the first location of the tissue sample into each of the ligated probes, or portions thereof, collected in step (c), thereby forming a first plurality of extension products that comprise the ligated probes, or portions thereof, collected in step (c) and the at least one nucleic acid sequence that identifies the first location of the tissue sample;
e) performing an extension reaction that incorporates at least one nucleic acid sequence that identifies the second location of the tissue sample into each of the nucleic acid probes, or portions thereof, collected in step (c), thereby forming a second plurality extension products that comprise the nucleic acid probes, or portions thereof, collected in step (c) and the at least one nucleic acid sequence that identifies the second location of the tissue sample; and	f) performing an extension reaction that incorporates at least one nucleic acid sequence that identifies the second location of the tissue sample into each of the ligated probes, or portions thereof, collected in step (d), thereby forming a second plurality extension products that comprise the ligated probes, or portions thereof, collected in step (d) and the at least one nucleic acid sequence that identifies the at least second location of the tissue sample; and

The challenged claims – identifying (sequencing) step

Claim 1	Claim 16
f) identifying the first plurality of extension products and the second plurality of extension products by sequencing the first plurality of extension products and the second plurality of extension products, thereby spatially detecting the at least one target analyte in the first location of the tissue sample and the second location of the tissue sample	g) identifying the first plurality of extension products and the second plurality of extension products by sequencing the first plurality of extension products and the second plurality of extension products, thereby spatially detecting the at least one target analyte in the first location of the tissue sample and the second location of the tissue sample.

Ground 4: Chell anticipates claims 1-30

Patent Owner does not dispute that Chell discloses all the elements of claims 1-30 under Bruker's interpretation, arranged as claimed

Patent Owner only disputes Chell's status as prior art POR, 40-59

PTAB:

Petitioner alleges that Chell discloses all limitations of challenged claims 1–30. Pet. 52–75. Patent Owner, for purposes of institution, does *[sic]* proffer any evidence to support its stated argument that Chell does not anticipate claims 1–30. *See, generally*, Prelim. Resp.

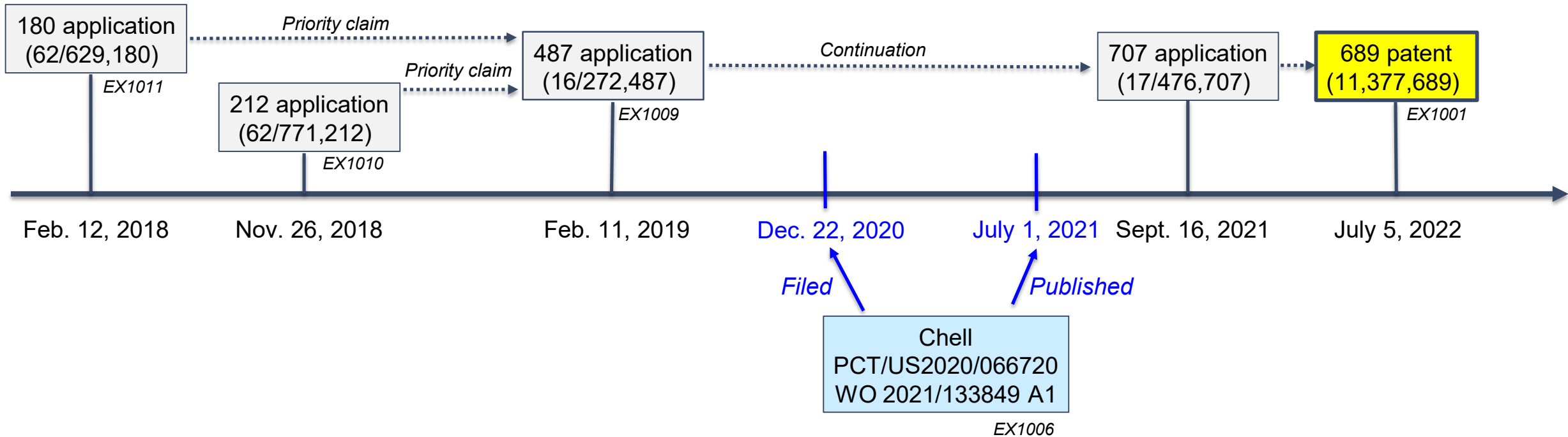
DOI, 36

Patent Owner:

Petitioner's ground 4 is based on the allegation that Chell anticipates the '689 patent claims. Chell, however, was not published until 2021 and is thus not prior art. Petitioner nonetheless argues that the '689 patent's priority application, the

POR, 40

Chell is intervening prior art because the 689 patent is not entitled to any of its claimed priority dates



The challenged claims reach beyond written description in the priority applications in two material ways

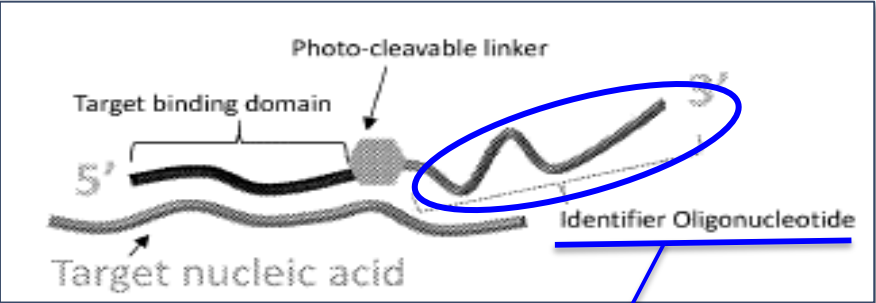
Scope of challenged claims	Written description in priority applications
Encompasses collecting whole probes	The specification of the priority applications only describes collecting portions of probes
Encompasses ligating pairs of nucleic acid probes in situ, before collection (on-analyte ligation)	The specification of the priority applications only describes off-analyte ligation

The priority applications do not describe collecting whole probes

The challenged claims encompass collecting whole probes

Claim 1	Claim 16
b) collecting the nucleic acid probes, or portions thereof , bound to the at least one target analyte in a first location of the tissue sample under conditions that release the nucleic acid probes, or portions thereof , from the first location of the tissue sample;	c) collecting the ligated probes, or portions thereof , bound to each of the identical molecules from the at least one target analyte in a first location of the tissue sample under conditions that release the ligated probes, or portions thereof , from the first location of the tissue sample;
c) collecting the nucleic acid probes, or portions thereof , bound to the at least one target analyte in a second location of the tissue sample under conditions that release the nucleic acid probes, or portions thereof , from the second location of the tissue sample;	d) collecting the ligated probes, or portions thereof , bound to each of the identical molecules from the at least one target analyte in a second location of the tissue sample under conditions that release the [ligated] probes, or portions thereof , from the second location of the tissue sample;

The priority applications uniformly describe collecting *only portions* of probes



EX1009, Fig. 13; POR, 48

1 Photoreleased Identifier Oligo

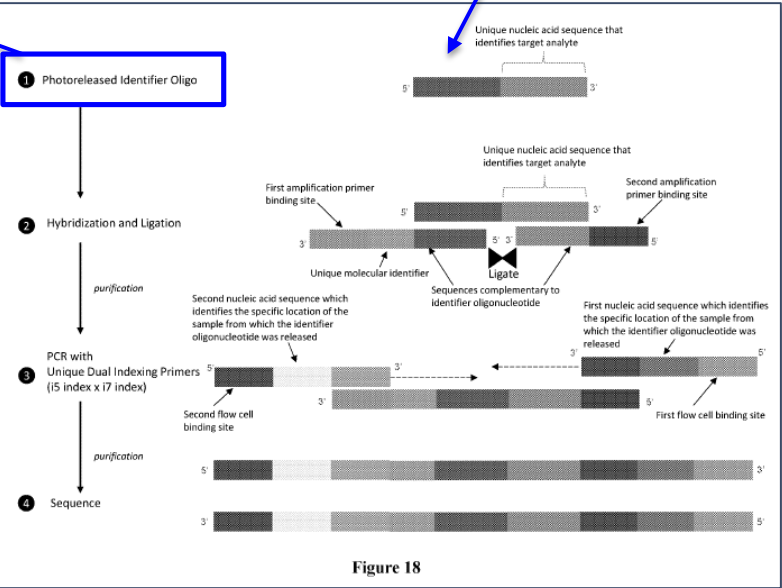


Figure 18

EX1009, Fig. 18

The Board agreed that all references to “collecting probes” refer to cleaved off identifier oligonucleotides



On the record before us, solely for purposes of institution, we find that the 487 application does not describe the use of whole/intact probes that are collected and extended as recited in the challenged claims. Rather, paragraphs 252 and 261 of the 487 application describe whole/intact probes floating in the in situ histochemistry milieu, and not bound to target analytes as the claims require. Ex. 1009 ¶¶ 252, 261. We conclude that, on this record, the disclosure of the 487 application describes whole probes only in the context of removing unbound whole probes to reduce signal noise. *Id.* As described above, all references to “collecting probes” refer only to cleaved off identifier oligonucleotides, not release of entire probes. Thus,

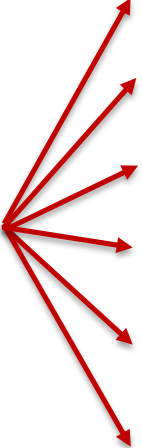
DOI, 35-36

Patent Owner attempts to stitch together various embodiments and unrelated disclosures to create written description

Patent Owner's relied-upon "disclosures"

Claim Element

Collecting whole probes

- 
- Specification's alleged disclosure of "**non-limiting**" **examples** of probe cleavage
 - Specification's disclosure of **denaturation** during **downstream cleanup for sequencing**
 - Disclosures in the 386 application regarding **methods of enzyme-free sequencing**
 - Specification's disclosure of **negative purification** to remove background signal
 - Specification's **silence** on target binding domain and identifier oligonucleotide being the same
 - Hypothetical indirect hybridization** embodiment with added cleavable motif

Patent Owner's argument that probe cleavage is "optional" is not supported in the 487 application's specification

Patent Owner:

cleavage to release the probe. EX2006 ¶¶ 92-93. Just the opposite, the specification explicitly describes embodiments of probes with a cleavable linker between the "identifier oligonucleotide" and the "target binding domain" as "a non-limiting example." See, e.g., EX1009 ¶¶ 106-107, 123-127, 131, 140-145, 159, 176, 180, 184, 200-201, 211, 221, 236, 246, 315-317. The '487 application further

POR, 43

All still require probe cleavage

The "non-limiting" examples in the 487 application refer to:

1. Specific **types of cleavage** (e.g., photocleavage)
2. Specific **target analytes** (e.g., P53, P97)
3. Aspects of workflow **after probe cleavage** (e.g., secondary probes)

Patent Owner's purported “**non-limiting example**” refers to the *type* of cleavage

Cleavage of the probe is still required

487 Appl.: [00107] Following contacting the at least one target analyte with the at least one probe, a two-ended adapter ligation method can further comprise: (2) providing a force to a location of the sample sufficient to release the identifier oligonucleotide. In a non-limiting example, in aspects in which the probe comprises a photo-cleavable linker between the identifier oligonucleotide and the target binding domain, a region-of-interest is excited with light of a sufficient wavelength capable of cleaving the photo-cleavable linker.

EX1009, ¶[00107]

Patent Owner's purported “**non-limiting example**” refers to *specific target analytes*

Cleavage of the probe is still required

487 Appl.: [00236] An identifier oligonucleotide is a nucleic acid molecule that identifies the target analyte bound to the target binding domain. The identifier oligonucleotide comprises a unique nucleic acid sequence that identifies the target analyte bound to the target binding domain of the probe. In a non-limiting example, a probe with a target binding domain that binds to the protein P53 comprises an identifier oligonucleotide with a unique nucleic acid sequence that corresponds to P53, while a probe with a target binding domain that binds to the protein P97 comprises an identifier oligonucleotide with a unique nucleic acid sequence that corresponds to P97.

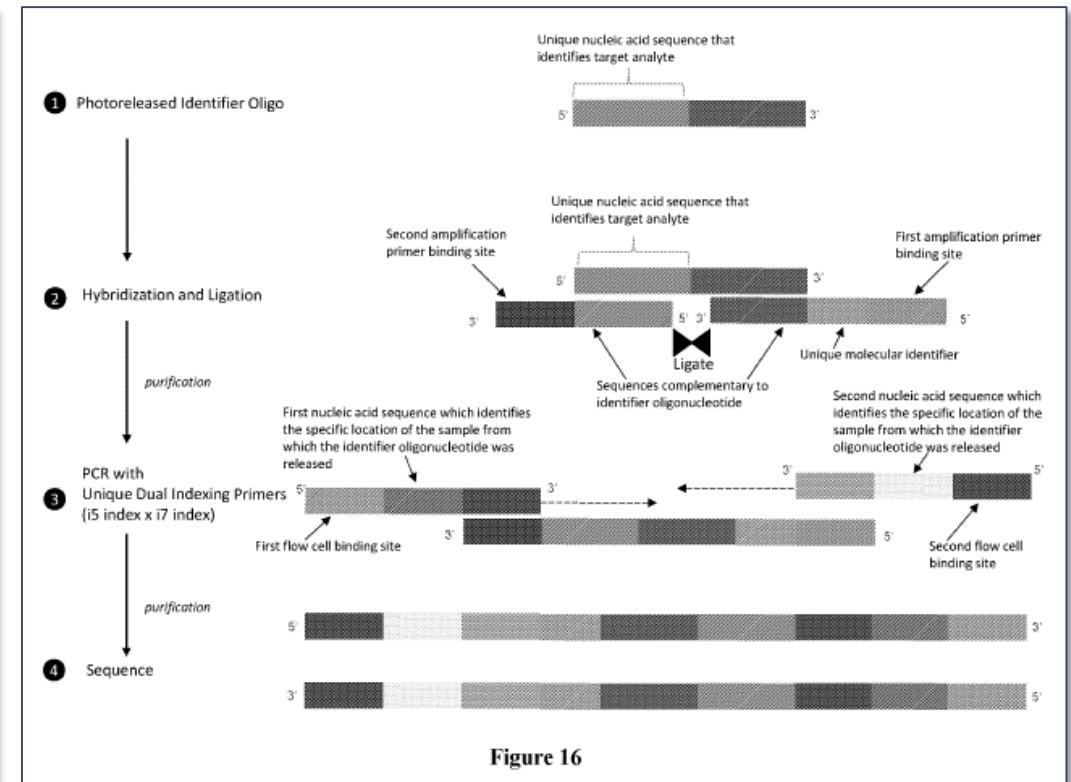
EX1009, ¶[00236]

Patent Owner's purported “**non-limiting example**” refers to aspects *after* cleavage of the probe has occurred

Cleavage of the probe is still required

487 Appl.:

[00176] As shown in the second panel from the top of Figure 16, the released identifier oligonucleotide is then hybridized to a first nucleic acid probe and a second nucleic acid probe. In this non-limiting example, the first nucleic acid probe comprises a first amplification primer binding site, a nucleic acid sequence comprising a unique molecular identifier and a nucleic acid sequence complementary to a portion of the identifier oligonucleotide. The nucleic acid sequence comprising the unique molecular identifier is located 3' to the first amplification primer binding site. The second nucleic acid probe comprises a second amplification primer binding site and a nucleic acid sequence complementary to a portion of the identifier oligonucleotide. In this non-limiting example, the first and the second nucleic acid probe hybridize to the identifier oligonucleotide such that the first and the second nucleic acid probes are adjacent and are not overlapping. Following hybridization to the identifier oligonucleotide, the first and second probe are ligated together, for example, by performing a nick repair reaction.



EX1009, ¶[00176]

EX1009, Fig. 16

Patent Owner's purported **denaturation** disclosure relates to downstream cleanup steps for sequencing

Patent Owner:

the target analyte through denaturation. As the '487 application teaches, “[s]uitable denaturing conditions will be apparent to the skilled reader with reference to standard molecular biology protocols.” EX1009 ¶ 285. And, during cross-

POR, 43

487 Appl.:

[00285] Following the cleavage step, regardless of the method used for cleavage, the product of the cleavage reaction may be subjected to denaturing conditions in order to remove the portion(s) of the cleaved strand(s) that are not attached to the solid support. Suitable denaturing conditions will be apparent to the skilled reader with reference to standard molecular biology protocols.

[00286] Denaturation (and subsequent re-annealing of the cleaved strands) results in the production of a sequencing template which is partially or substantially single-stranded. A sequencing reaction may then be initiated by hybridization of a sequencing primer to the single-stranded portion of the template.

EX1009, ¶¶[00285]-[00286]

The 386 application's purported incorporation relates to methods of enzyme-free sequencing

Enzyme-free sequencing occurs *after* cleavage of the probe

487 Appl.:

[00213] Following collection of a released identifier oligonucleotide, an enzyme free method can further comprise: (4) hybridizing to a released identifier oligonucleotide a capture probe.

EX1009, ¶[00213]

[00216] A hybridized product produced in step (4) can be sequenced using an enzyme free method of sequencing. Enzyme-free methods of sequencing have been described in, e.g., US2014946386 and US15/819,151, each of which is incorporated herein by reference in its entirety.

Unclear identification

EX1009, ¶[00216]; see also, ¶[00227]

The 386 application does not disclose collecting whole probes from the target analyte in a tissue sample

The 386 application does not describe using denaturation to collect whole probes in the claimed methods

386 Appl.:

[0010] Provided herein is a method for sequencing a nucleic acid using a sequencing probe of the present invention. The method comprises steps of: (1) hybridizing at least one sequencing probe, of the present invention, to an target nucleic acid that is immobilized (e.g., at one, two, three, four, five, six, seven, eight, nine, ten or more positions) to a substrate; (2) binding to the first attachment region a first complementary nucleic acid molecule (RNA or DNA) which has a detectable label (e.g., a fluorescent label) or a first complementary nucleic acid molecule of a first reporter complex comprising detectable labels (e.g., fluorescent labels); (3) detecting the detectable label(s), and (4) identifying the position and identity of the first nucleotide in the immobilized target nucleic acid. Optionally, the immobilized target nucleic acid is elongated prior to being bound by the probe. The method further comprises steps of: (5) contacting the first attachment region (with or without one or two flanking single-stranded polynucleotides) with a first hybridizing nucleic acid molecule lacking a detectable label, thereby unbinding the first complementary nucleic acid molecule having a detectable label or the first complementary nucleic acid molecule of a first reporter complex comprising detectable labels and binding to, at least, the first attachment region a first hybridizing nucleic acid lacking a detectable label; (6) binding to the second attachment region a second complementary nucleic acid molecule having a detectable label or a complementary nucleic acid molecule of a second reporter complex comprising detectable labels; (7) detecting the detectable label(s); and (8) identifying the position and identity of the second nucleotide in the immobilized target nucleic acid. Steps (5) to (8) are repeated until each nucleotide in the immobilized target nucleic acid and corresponding to the target binding domain has been identified. Steps (5) and (6)

POR, 44; EX2008, ¶[0010]

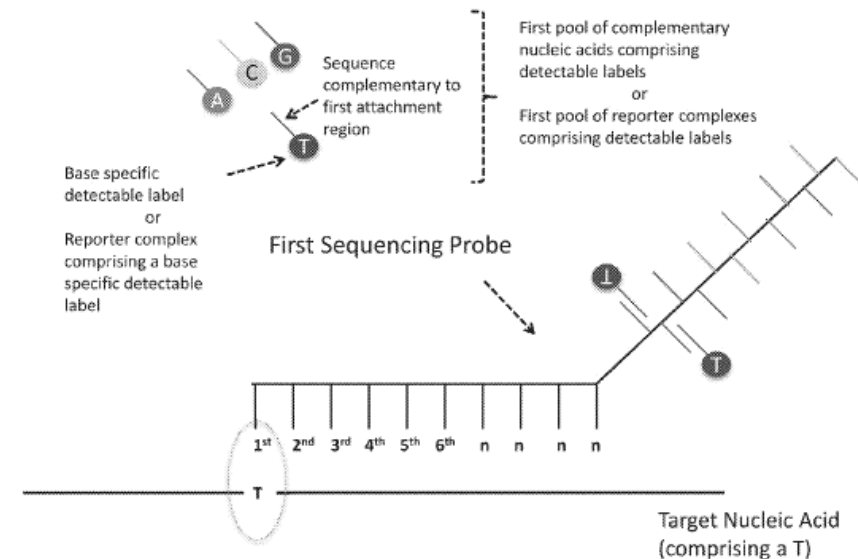
386 Appl.:

Example 1

The Present Invention's Method of Sequencing a Target Nucleic Acid is Rapid

POR, 45; EX2008, ¶[0174]-[0194]

Figure 8A



EX2008, Fig. 8A

The 487 application's **paragraph 240** discloses denaturing for improving release of identifier oligonucleotides **after cleavage**

Cleavage of the probe is still required

487 Appl.:

[00240] An identifier oligonucleotide can be a single-stranded, a double-stranded, or a partially double-stranded nucleic acid molecule. In the aspects in which an identifier oligonucleotide is double-stranded or partially double-stranded, at least one of the two strands can comprise at least two separate nucleic acid molecules which, without being bound by theory, allows for denaturing of the identifier oligonucleotide at lower temperatures.

EX1009, ¶[00240]

Dr. Spellman:

“Having a double-stranded identifier oligonucleotide portion of the probe prevents the identifier oligonucleotide from hybridizing non-specifically with the target analyte. This reduces the amount of non-specific hybridization between the identifier oligonucleotide and the target analyte, improving efficient release of the identifier oligonucleotide portion ... The whole probes are not being released by denaturation.”

EX1055, ¶95

The 487 application's **paragraph 259** is a generic reference to “any probe” and is limited by paragraph 64 “conflicts”

487 Appl.:

[00259] Any probe or set of probes described herein may be used in methods and kits of the present disclosure.

EX1009, ¶[00259]

described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. The references cited herein are not admitted to be prior art to the claimed invention. In the case of conflict, the present Specification, including definitions, will control. In addition, the materials, methods, and examples are

EX1009, ¶[0064]

The 487 application's **negative purification** does not collect whole probes, as claimed

487 Appl.:

[00252] Background noise, during nucleic acid detection, can be reduced by performing a negative purification of the intact probe molecule. This can be done by conducting an affinity purification of the target binding domain or photo-cleavable linker after collection of eluate from a region of interest. Normally, released signal oligonucleotides will not be pulled out of solution. To assist in the negative purification, a universal purification sequence may be included in a probe, e.g., in the target binding domain.

EX1009, ¶[00252]

Background noise caused by inefficient binding and inefficient cleavage

Uncleaved probes collected by negative purification are **discarded** to reduce background noise

Dr. Sherman:

Q. What would -- generally, what would the POSA's understanding of the term "negative purification" be?

A. To **remove some sort of analyte from a mixture.**

EX1056, 60:22-24

Dr. Sherman:

Q. And do you agree that this is a disclosure stating that background noise can be reduced by pulling out the [intact] probe molecule?

A. **Yes, I agree.**

EX1056, 61:24-62:2

The 487 application's **negative purification** does not collect whole probes, as claimed

PTAB:

On the record before us, solely for purposes of institution, we find that the 487 application does not describe the use of whole/intact probes that are collected and extended as recited in the challenged claims. Rather, paragraphs 252 and 261 of the 487 application describe whole/intact probes floating in the in situ histochemistry milieu, and not bound to target analytes as the claims require. Ex. 1009 ¶¶ 252, 261. We conclude that, on this record, the disclosure of the 487 application describes whole probes only in the context of removing unbound whole probes to reduce signal noise. *Id.*

DOI 35-36



No disclosure of collecting **and extending** whole, uncleaved probes



Dr. Sherman:

“In both claims [1 and 16], an **extension, or amplification** step, is **performed on the collected probes**.”

EX2006, ¶38

Pet. Reply, 6-7; EX1055, ¶¶104-110

The 487 application does not describe probes with target binding domain and identifier oligonucleotide being the **same or overlapping**

Patent Owner:

As additional support for the release of “whole probes,” the ‘487 application never states that the “target-binding domain” and “identifier oligonucleotide” portions of a probe cannot overlap or even be the same sequence. The specification describes both portions in functional terms: the “target-binding domain” is a sequence that “can directly or indirectly hybridize to a target nucleic acid of interest” and an “identifier oligonucleotide” is a sequence “which identifies the target analyte bound to the target binding domain.” EX1009 ¶¶ 106, 126, 144, 158, 183, 199. Nothing forecloses the same sequence from serving both functions. Accordingly,

POR, 45-46

487 Appl.:

a target protein-binding region that can specifically bind to a target protein of interest. In the aspect that the at least one target analyte is a target nucleic acid, the probe can comprise a target nucleic acid-binding region that can directly or indirectly hybridize to a target nucleic acid of interest. The probe further comprises an identifier oligonucleotide. The identifier oligonucleotide

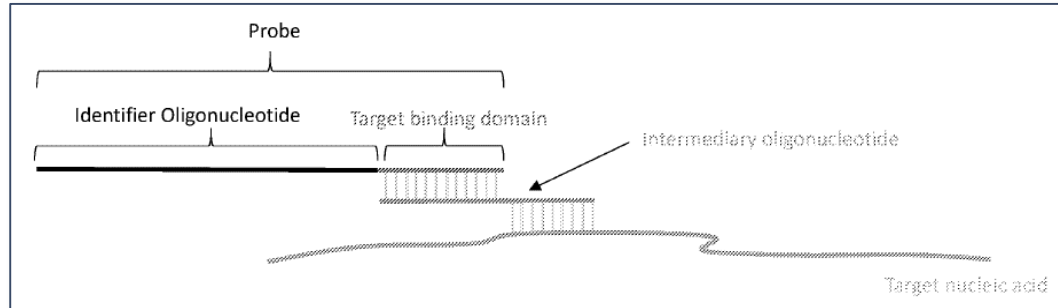
EX1009, ¶[00106]

that is a target protein-binding region that can specifically bind to a target protein of interest. In the aspect that the at least one target analyte is a target nucleic acid, the probe can comprise a target nucleic acid-binding region that can directly or indirectly hybridize to a target nucleic acid of interest. The probe further comprises an identifier oligonucleotide. The identifier

EX1009, ¶[00144]; see also, ¶¶[00126], [00158], [00183], [00199]

Patent Owner's hypothetical **indirect hybridization** embodiment is not described

487 Appl.:



EX1009, Fig. 14



“[A]dequate written description does **not ask what is permissible**, rather, it **asks what is disclosed.**”

D Three Enterprises, LLC v. SunModo Corp., 890 F.3d 1042, 1052 (Fed. Cir. 2018)

Dr. Sherman:

“a POSA would have understood that an ‘intermediary oligonucleotide’ **can contain** ‘a region which permits the release of an identifier oligonucleotide,’ including ‘a cleavable motif’” such that “it **would** lead to **release** of an entire probe.”

EX2006, ¶103; see also POR, 47-48

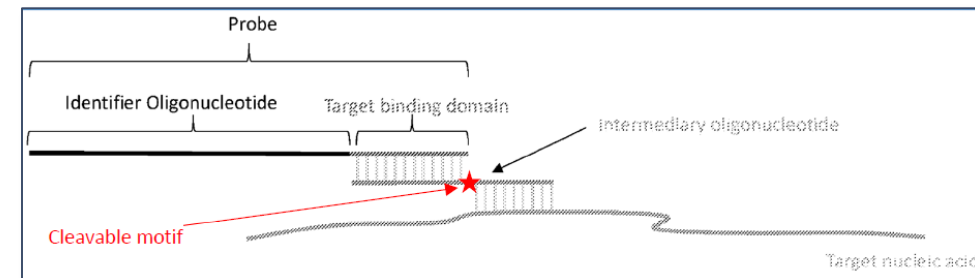
“Such an arrangement **can be as depicted** as follows....”

EX2006, ¶103; see also POR, 47-48

Patent Owner:

“This is depicted in a **modified version** of Figure 14....”

PO Sur-Reply, 6



POR, 47; EX2006, ¶103

Pet. Reply, 6, 8-9; EX1055, ¶¶115-123

None of the 487 application's **indirect hybridization** disclosures include a cleavable motif in the intermediary oligonucleotide

487 Appl.:

a target protein-binding region that can specifically bind to a target protein of interest. In the aspect that the at least one target analyte is a target nucleic acid, the probe can comprise a target nucleic acid-binding region that can directly or indirectly hybridize to a target nucleic acid of interest. The probe further comprises an identifier oligonucleotide. The identifier oligonucleotide can comprise a unique nucleic acid sequence which identifies the target analyte bound to the target binding domain.

[00159] Following contacting the at least one target analyte with the at least one probe, a short probe hybridization method can further comprise: (2) providing a force to a location of the sample sufficient to release the identifier oligonucleotide. In a non-limiting example, in aspects in which the probe comprises a photo-cleavable linker between the identifier oligonucleotide and the target binding domain, a region-of-interest (ROI) is excited with light of a sufficient wavelength capable of cleaving the photo-cleavable linker.

All of the 487 application's indirect hybridization disclosures include a cleavable motif between the target binding domain and identifier oligonucleotide

see also, EX1009, ¶¶[0080], [00106]-[00108], [00126]-[00128], [00144]-[00146], [00158]-[00160], [00174]-[00176], [00178]-[00179], [00183]-[00185], [00199]-[00202], [00210]-[00212], [00220]-[00222], [00233], [00310], [00420], FIGs. 14, 26, 27

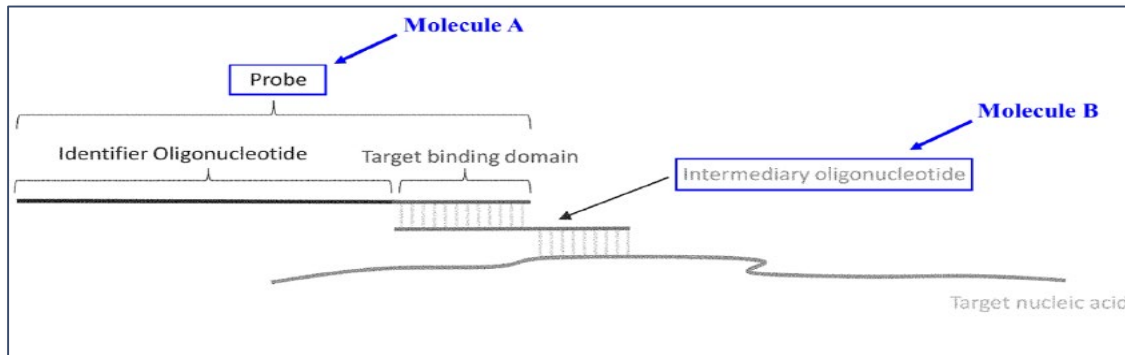
EX1009, ¶¶[00158]-[00159]

Dr. Spellman explains: Patent Owner's **hypothetical embodiment** would eliminate the 487 application's stated advantages

487 Appl.:

sample. This allows **economical and rapid flexibility** in an assay design, as **the target (present in a sample)-specific components** of the assay are included in **inexpensive and widely-available synthetic DNA oligonucleotides** rather than the **more expensive probes**. Such synthetic oligonucleotides are simply designed by including a region that hybridizes to the target nucleic acid present in a sample and a region that hybridizes to a probe. Therefore, a **single set of indirectly-binding probes** can be used to detect an infinite variety of target nucleic acids (present in a sample) in different experiments simply **by replacing the target-specific (synthetic) oligonucleotide portion** of the assay.

EX1009, ¶¶00233]



EX1055, ¶117

Dr. Spellman:

“As a result, indirect hybridization under Dr. Sherman’s scheme **would become more expensive and removes the advantages**, defeating the whole purpose of indirect hybridization stated in the 487 application.

Furthermore, a **cleavable Molecule B would render Molecule A redundant** ... Thus, there would be no need for an additional probe (Molecule A) that comprises a target binding domain and an identifier oligonucleotide if the method already includes a cleavable Molecule B.”

EX1055, ¶¶120-121

Q Does it states in Paragraph 233 why the probes are more expensive?

MS. KHANDURI: Objection. Scope.

Relevance.

A Explicitly, no. It implies that the differences between what the probes are and the synthetic DNA oligonucleotides, the indirect hybridization pieces, **the principal difference between them is the cleavable linker.**

Obviousness is insufficient for written description



“[I]t is **the specification itself that must demonstrate possession**. And while the description requirement does not demand any particular form of disclosure ... or that the specification recite the claimed invention in haec verba, **a description that merely renders the invention obvious does not satisfy the requirement.**”

Ariad Pharms., Inc. v. Eli Lilly and Co., 598 F.3d 1336, 1352 (Fed. Cir. 2010) (en banc)

“[A]dequate written description does **not ask what is permissible**, rather, it **asks what is disclosed.**”

D Three Enterprises, LLC v. SunModo Corp., 890 F.3d 1042, 1052 (Fed. Cir. 2018)

“To the extent that Purdue contends that a [POSA] would isolate and combine **aspects from various embodiments** in the specifications (**including patents incorporated by reference** involving a different drug) to obtain the claimed invention, Purdue **relies upon the wrong test** [for written description].”

Purdue Pharma, LP v. Recro Tech. LLC, 694 Fed.Appx. 794, 797 (Fed. Cir. 2017) (non-precedential)

Pet. Reply, 6, 8, 12; EX1055, ¶¶104-110, 116-118, 133-137

**The priority applications do not describe
ligation before collection**

Claims 16-30 require probe ligation before collection (on-analyte)

16. A method for spatially detecting at least one target analyte in a first location and a second location of a tissue sample comprising:

a) contacting the tissue sample with a first plurality of nucleic acid probes and a second plurality of nucleic acid probes,

wherein each of the nucleic acid probes in the first plurality of nucleic acid probes comprise a target binding domain that binds to the at least one target analyte,

wherein each of the nucleic acid probes in the second plurality of nucleic acid probes comprise a target binding domain that binds to the at least one target analyte,

wherein the tissue sample is treated to facilitate binding of the first plurality of nucleic acid probes and the second plurality of nucleic acid probes to the at least one target analyte;

b) forming ligated probes by ligating each of nucleic acid probes from the first plurality of nucleic acid probes and each of nucleic acid probes from the second plurality of nucleic acid probes that are bound to each of identical molecules from the at least one target analyte;

c) collecting the ligated probes, or portions thereof, bound to each of the identical molecules from the at least one target analyte in a first location of the tissue sample under conditions that release the ligated probes, or portions thereof, from the first location of the tissue sample;

d) collecting the ligated probes, or portions thereof, bound to each of the identical molecules from the at least one target analyte in a second location of the tissue sample under conditions that release the probes, or portions thereof, from the second location of the tissue sample;

e) performing an extension reaction that incorporates at least one nucleic acid sequence that identifies the first location of the tissue sample into each of the ligated probes, or portions thereof, collected in step (c), thereby forming a first plurality of extension products that comprise the ligated probes, or portions thereof, collected in step (c) and the at least one nucleic acid sequence that identifies the first location of the tissue sample;

f) performing an extension reaction that incorporates at least one nucleic acid sequence that identifies the second location of the tissue sample into each of the ligated probes, or portions thereof, collected in step (d), thereby forming a second plurality extension products that comprise the ligated probes, or portions thereof, collected in step (d) and the at least one nucleic acid sequence that identifies the at least second location of the tissue sample; and

g) identifying the first plurality of extension products and the second plurality of extension products by sequencing the first plurality of extension products and the second plurality of extension products, thereby spatially detecting the at least one target analyte in the first location of the tissue sample and the second location of the tissue sample.

b) forming ligated probes by **ligating** each of nucleic acid probes from the first plurality of nucleic acid probes and each of nucleic acid probes from the second plurality of nucleic acid probes **that are bound to each of identical molecules from the at least one target analyte;**

c) collecting the **ligated probes**, or portions thereof, **bound to each of the identical molecules from the at least one target analyte** in a first location of the tissue sample under conditions that release the ligated probes, or portions thereof, from the first location of the tissue sample;

d) collecting the **ligated probes**, or portions thereof, **bound to each of the identical molecules from the at least one target analyte** in a second location of the tissue sample under conditions that release the probes, or portions thereof, from the second location of the tissue sample;

EX1001, claim 16

The priority applications uniformly describe only performing **ligation after collection** in the claimed methods

487 Appl.:

[0024] The present disclosure provides a method for spatially detecting at least one target analyte in at least one cell from a tissue sample comprising: (1) contacting at least one target analyte in at least one cell in a tissue sample with at least **one probe comprising a target binding domain and an identifier oligonucleotide**, wherein the identifier oligonucleotide comprises a unique nucleic acid sequence which identifies the target analyte bound to the target binding domain; (2) providing a force to a location of the tissue sample sufficient to **release the identifier oligonucleotide**; (3) **collecting** the released identifier oligonucleotide; (4) **hybridizing** to the released identifier oligonucleotide a **first nucleic acid probe and a second nucleic acid probe**,

and the second nucleic acid probes are adjacent but not overlapping; (5) performing nick repair such that the hybridized **first and second nucleic acid probes are ligated together**; (6) amplifying the ligation product produced in step (5); and (7) **Identifying the released identifier oligonucleotide by sequencing** the amplified products produced in step (6), thereby spatially detecting the at least one target analyte in the at least one cell in a tissue sample.

On-analyte steps:

- (1) Contacting with cleavable primary “probes”
- (2) Providing “force” to cleave and release identifier oligonucleotides

Off-analyte steps:

- (3) Collecting cleaved identifier oligonucleotides off tissue
- (4) Adding pairs of secondary “nucleic acid probes” that hybridize to identifier oligonucleotides
- (5) **Ligating first and second nucleic acid probes**
- (6) Amplifying ligated probe product
- (7) Sequencing the amplified products

EX1009, ¶[0024]

Pet, 55-57; EX1002, ¶¶287-292; EX1009, ¶¶[0005]-[0007], [0014]-[0015], [0021]-[0022], [0024]-[0027], [0033]-[0036], [0043]-[0048], [00106], [00123], [00126], [00140], [00144], [00155], [00158], [00174], [00178], [00183], [00199], [00210], [00220], [00228], [00231]-[00232], [00235]-[00236], [00246], [00310], [00315], [00317], [00409], [00411], [00413], [00415]

Description of the accused product is not the issue – the specification must describe the full scope of what is claimed

Patent Owner:

Petitioner’s attempt to measure the adequacy of written description based on whether the ’487 specification discloses Petitioner’s accused product is unsupported in law and is reason enough to reject its written description arguments.

POR, 51

But see:



For written description, the specification must “sufficiently demonstrate[] that the **inventors possessed the full scope of the claimed invention.**”

Juno Therapeutics, Inc. v. Kite Pharma, Inc., 10 F.4th 1330, 1336 (Fed. Cir. 2021)

For written description, the specification must “demonstrate that the **inventor possesses the full scope of the invention.**”

LizardTech, Inc. v. Earth Resource Mapping, Inc., 424 F.3d 1336, 1345 (Fed. Cir. 2005)

“The fact finder must determine if one skilled in the art, reading the original specification, would **immediately discern the limitation at issue** in the parent.”

Waldemar Link GmbH & Co. v. Osteonics Corp., 32 F.3d 556, 558 (Fed. Cir. 1994)

Patent Owner attempts to stitch together various embodiments to create written description

Patent Owner's relied-upon "disclosures"

Claim Element

On-analyte ligation

- Disclosures in the **607 publication** regarding nanoreporter probes that are **not ligated together**
- Specification's **indirect hybridization** embodiment in which probes are **not ligated together**
- Disclosures in the **774 publication** regarding proximity ligation in which **portions** of probes are collected
- Disclosures in the **111 publication** which **does not** provide **written description** in the 487 application

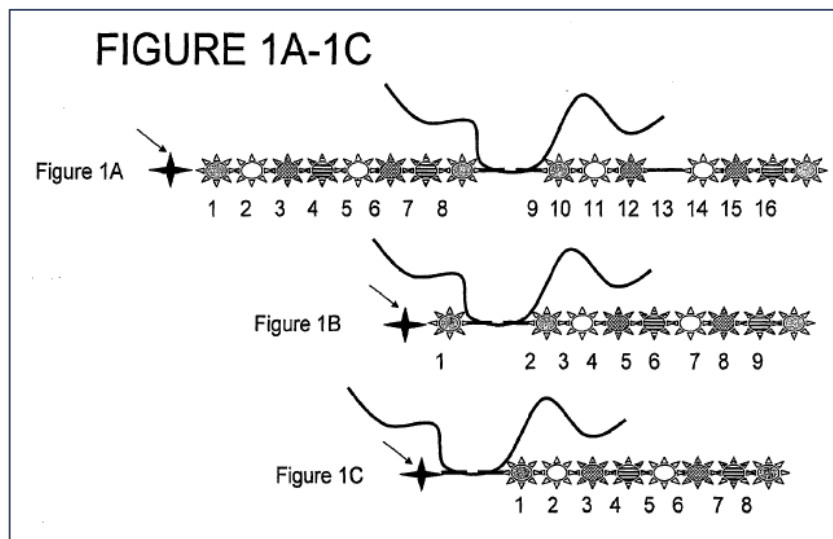
The **607 publication** does not provide written description of probe ligation before collection, as claimed

Patent Owner:

“[P]atent application publication no. 2010/0015607 ... teaches **two probes that hybridize to a target analyte.**”

POR, 51-52; EX2009, ¶[0013], Fig. 1A-1C

607 publication:



EX2009, FIG. 1A-1C

No disclosure of
on-analyte
ligation

Patent Owner
does not argue
otherwise

607 publication:

[0013] In alternative embodiments, the present invention provides a **dual nanoreporter, or a “probe pair,”** comprising two components: a first probe and a second probe. In specific embodiments, **the first probe** is a complex comprising: (a) a first molecule, or a scaffold, comprising: (i) a first label attachment region to which are attached (directly or indirectly) one or more label monomers that emit light constituting a first signal; (ii) a second label attachment region to which are attached (directly or indirectly) one or more label monomers that emit light constituting a second signal; and (b) a first target-specific sequence attached to the first molecule. **The second probe** is a second molecule or a complex comprising a second molecule, said second molecule comprising (i) a second target-specific sequence; (ii) optionally, a third label attachment region to which are attached (directly or indirectly) one or more label monomers that emit light constituting a third signal; and (iii) optionally, an affinity tag attached to said second molecule; wherein the first signal and the second signal are spatially or spectrally distinguishable; wherein the first target-specific sequence and the second target-specific sequence bind to different regions of the same target molecule and wherein **when said probe pair is bound to its target molecule, the identity of the first and second signals and their locations relative to each other constitutes at least part of a code that identifies the target molecule.**

Combination of the 607 publication with indirect hybridization has no ligation of probe pairs, let alone ligation before collection

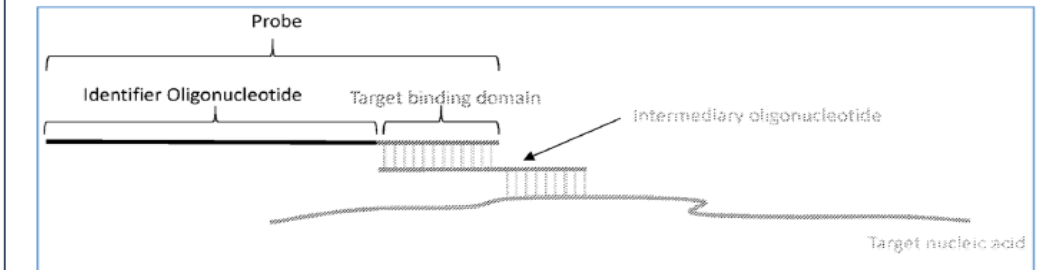
Dr. Sherman:

111. The '607 Publication teaches that a probe pair that can hybridize to a target nucleic, wherein a first probe comprises "a first target-specific sequence attached to the first molecule" and a second probe comprises a "second target-specific sequence." EX2009 ¶ 13. The '607 Publication teaches that these

EX2006, ¶111

+

EX1009 ¶ 233. A POSA would have understood that a probe pair can indirectly hybridize to a target nucleic acid via an indirect target binding domain. An example of this concept is depicted in Figure 14:



EX2006, ¶112; POR, 52-53

Dr. Sherman: **no ligation** in this hypothetical embodiment

Q. If this intermediary oligonucleotide is a pair of probes or a probe pair, as you've said, is it subsequently ligated to another probe?

A. In this case, there is no need for that.

EX1056, 68:10-13

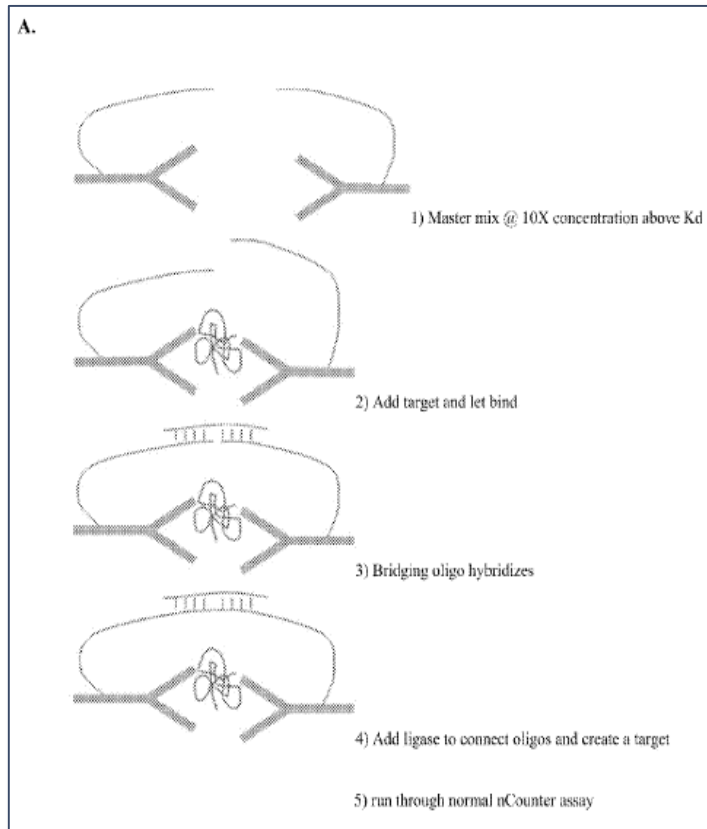
Q. So this would be a -- in your view, this is a probe pair that's not ligated together after binding the target nucleic acid?

A. There's no need for it because the probe pair, as you see, is -- comprises this intermediary oligonucleotide with two hybridizing regions, so there's no need for a ligase or a ligation.

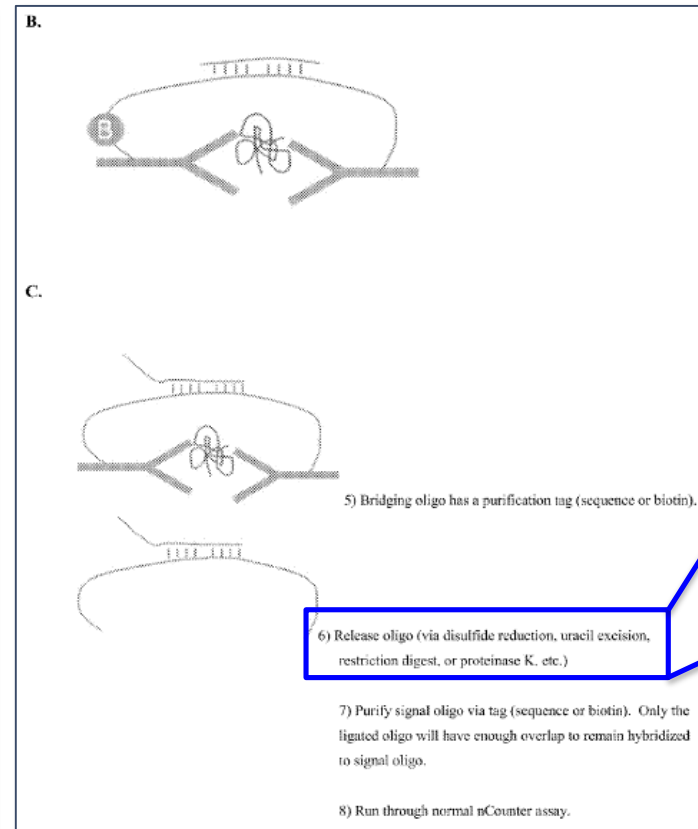
EX1056, 68:14-20

The 774 publication's proximity ligation methods do not support written description of the claimed methods

774 publication: release and collection of portions of probes



EX2010, Fig. 8A



EX2010, Fig. 8B-8C

[0086] In step 5) of FIG. 8, after ligation, the signal oligo is then released via disulfide reduction, uracil excision, restriction digest, proteinase K, or any other suitable method known in the art. Additionally, the signal oligo can be released by the methods depicted in FIG. 8B-8D. FIG. 8B, shows an embodiment of the methods depicted in FIG. 8B-8D.

EX2010, ¶[0086]

6) Release oligo (via disulfide reduction, uracil excision, restriction digest, or proteinase K, etc.)

Bruker misrepresents the 111 publication as part of the 689 patent disclosures

Patent Owner:

The longstanding and fundamental nature of ligation of probes is clear from Petitioner's own patents. The '689 patent discloses Petitioner's patent publication No. 2011/0245111, a prior art reference that Petitioner acquired from a company called Prognosys. EX2011 at 208. There, Petitioner's patent application

POR, 56

Dr. Spellman:

139. As is clear from my testimony, I do not dispute that ligation *generally* is a simple skill. *See also* EX2007, 93:4-10 ("The *abstract concept of ligation*, surely understood to a POSA, yes."). But whether or not ligation is a simple skill is not the issue here. The issue here is the 487 application's failure to describe to a POSA the claimed methods comprising "on-analyte" ligation before collection.

EX1055, ¶139

The 111 publication is **disclosed in an IDS**, and is **not** part of the 689 patent's written description

SHEET 4 OF 9

Substitute for form 1449/PTO INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use as many sheets as necessary)	Complete if Known	
	Application Number	17476,707
	Filing Date	09-16-2021
	First Named Inventor	Joseph M. BEECHEM
	Art Unit	1634
	Examiner Name	Not Yet Assigned
Attorney Docket Number	NATE-037/C01US 321329-2719	

U.S. PATENT DOCUMENTS					
Examiner Initials*	Cite No. ¹	Document Number Number-Kind Code ² (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	67.	2010/0015607	01-21-2010	Geiss et al.	
	68.	2010/0047924	02-25-2010	Webster et al.	
	69.	2010/0075858	03-25-2010	Davis et al.	
	70.	2010/0112710	05-06-2010	Geiss et al.	
	71.	2010/0151472	06-17-2010	Nolan et al.	
	72.	2010/0209913	08-19-2010	Endress et al.	
	73.	2010/0261026	10-14-2010	Ferree et al.	
	74.	2010/0262374	10-14-2010	Hwang et al.	
	75.	2011/0086774	04-14-2011	Dunaway et al.	
	76.	2011/0151451	06-23-2011	Lemaire et al.	
	77.	2011/0172115	07-14-2011	Thompson	
	78.	2011/0223613	09-15-2011	Gut	
	79.	2011/0245111	10-06-2011	Chee	
	80.	2013/0023433	01-24-2013	Luo et al.	
	81.	2013/0178372	07-11-2013	Geiss et al.	
	82.	2014/0120532	05-01-2014	Lee et al.	
	83.	2014/0120550	05-01-2014	Baranov	
	84.	2014/0121117	05-01-2014	Tanner	
	85.	2014/0371088	12-18-2014	Webster	
	86.	2015/0080233	03-19-2015	Bendall et al.	
	87.	2015/0099650	04-09-2015	Sood et al.	
	88.	2015/0132763	05-14-2015	Amorese et al.	

EX2011, 208

The claimed steps have a required order that the specification does not describe

Patent Owner:

analyte, as disclosed by the application, prior to ligation. EX2006 ¶ 115. To the extent Petitioner argues that the ligation must occur while the probe pair is hybridized to the target analyte, a POSA would have understood that this is disclosed as well, since the specification does not require disclosed steps to take place in order.

POR, 54

689 patent, claim 16:

- b) forming ligated probes by ligating each of nucleic acid probes from the first plurality of nucleic acid probes and each of nucleic acid probes from the second plurality of nucleic acid probes that are bound to each of identical molecules from the at least one target analyte;
- c) collecting the ligated probes, or portions thereof, bound to each of the identical molecules from the at least one target analyte in a first location of the tissue sample under conditions that release the ligated probes, or portions thereof, from the first location of the tissue sample;
- d) collecting the ligated probes, or portions thereof, bound to each of the identical molecules from the at least one target analyte in a second location of the tissue sample under conditions that release the probes, or portions thereof, from the second location of the tissue sample;

EX1001, claim 16



“[A] claim requires an ordering of steps when the claim language, as a **matter of logic or grammar**, requires that **the steps be performed in the order written....**”

Mformation Techs. v. Research in Motion, 764 F.3d 1392, 1398 (Fed. Cir. 2014)

Pet. Reply, 13-15; EX1055, ¶¶143-145

Every ligation embodiment in the 487 application requires the same order of steps

Dr. Spellman:

“require” the steps to be in a specific order is incorrect. A POSA would have readily understood that every ligation-based embodiment in the 487 application requires the same order of steps: collecting the released identifier oligonucleotides (off analyte); hybridizing secondary probe pairs to the collected identifier oligonucleotides (off analyte); and ligating the secondary probe pairs (off analyte). EX1009, ¶¶[0024]-[0027], [0033]-[0036], [0047], [00161], [00168]-[00169], [00174]-[00180], [00183]-[00186], [00389]; *see also* EX1002, ¶¶287-292.

EX1055, ¶145

The facts here align with *ICU Medical*, not *Allergan*

Patent Owner:

be supported by the '487 application. Instructive on this issue is the Federal Circuit's recent decision in *Allergan*, which shows that Petitioner's contention regarding written description are without merit.

POR, 58

Allergan USA, Inc. v. MSN Lab'ys Priv. Ltd.

Issue: does specification describe composition that *lacked* a "glidant?"

Answer: **Yes**, because

- Specification described **at least two embodiments** in which a glidant was not required; and
- The **original claims** in priority application recited **glidant-optional** compositions

Allergan USA, Inc. v. MSN Lab'ys Priv. Ltd., 111 F.4th 1358, 1373 (Fed. Cir. 2024)

487 application

Issue: does specification describe on-analyte ligation *before collection*?

Answer: **No**, because

- **No examples** in the 487 application with ligation optionally occurring **before collection**; and
- All **original claims** in priority applications require ligation **after collection**
- Specification "**repeatedly and uniformly**" described only ligation **after collection**

ICU Medical v. Alaris Medical Sys.

Issue: does specification describe valves that *lack* a spike (spikeless valves)?

Answer: **No**, because

- Specification "**repeatedly and uniformly**" described **only spiked valves**

ICU Medical v. Alaris Medical Systems, 558 F.3d 1368, 1375-1376 (Fed. Cir. 2009); see also *D Three Enterprises, LLC v. SunModo Corp.*, 890 F.3d 1042, 1052 (Fed. Cir. 2018)

Patent Owner's stitched-together embodiments and disclosures are *obviousness* arguments, not written description

Patent Owner's relied-upon "disclosures"

Claim Element

Collecting whole probes

- Allegation that cleavage is "optional" due to **"non-limiting"** examples
- Specification's disclosure of **denaturation** during **downstream cleanup for sequencing**
- Disclosures in the 386 application regarding **methods of enzyme-free sequencing**
- Specification's disclosure of **negative purification** to remove background signal
- Specification's **silence** on target binding domain and identifier oligonucleotide being the same
- Hypothetical indirect hybridization** embodiment with added cleavable motif

On-analyte ligation

- Disclosures in the **607 publication** regarding nanoreporter probes that are **not ligated together**
- Specification's **indirect hybridization** embodiment in which probes are **not ligated together**
- Disclosures in the **774 publication** regarding proximity ligation in which **portions** of probes are collected
- Disclosures in the **111 publication** which **does not** provide **written description** in the 487 application

Obviousness is insufficient for written description



“[I]t is **the specification itself that must demonstrate possession**. And while the description requirement does not demand any particular form of disclosure ... or that the specification recite the claimed invention in haec verba, **a description that merely renders the invention obvious does not satisfy the requirement.**”

Ariad Pharms., Inc. v. Eli Lilly and Co., 598 F.3d 1336, 1352 (Fed. Cir. 2010) (en banc)

“[A]dequate written description does **not ask what is permissible**, rather, it **asks what is disclosed.**”

D Three Enterprises, LLC v. SunModo Corp., 890 F.3d 1042, 1052 (Fed. Cir. 2018)

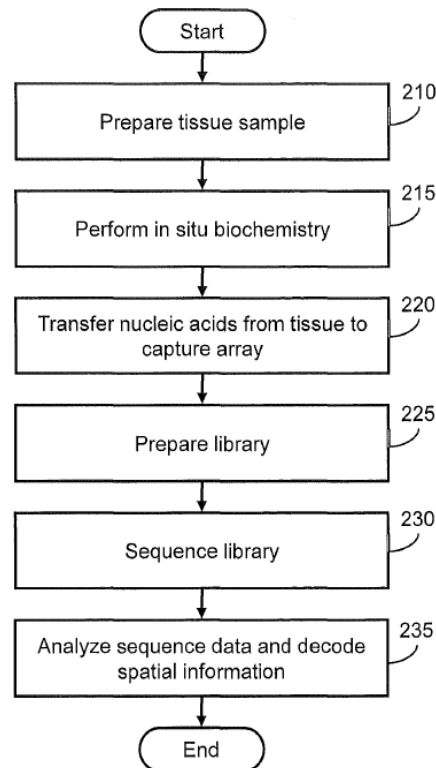
“To the extent that Purdue contends that a [POSA] would isolate and combine **aspects from various embodiments** in the specifications (**including patents incorporated by reference** involving a different drug) to obtain the claimed invention, Purdue **relies upon the wrong test** [for written description].”

Purdue Pharma, LP v. Recro Tech. LLC, 694 Fed.Appx. 794, 797 (Fed. Cir. 2017) (non-precedential)

Ground 1: So-456 anticipates claims 1-8, 11-23, and 26-30

So-456's FIGs. 8-9 method includes all claim elements under Bruker's infringement interpretation

FIG. 2:
Overall workflow of So-456's methods



EX1005, Fig. 2

FIGs. 8-9:
So-456's method applying FIG. 2's workflow

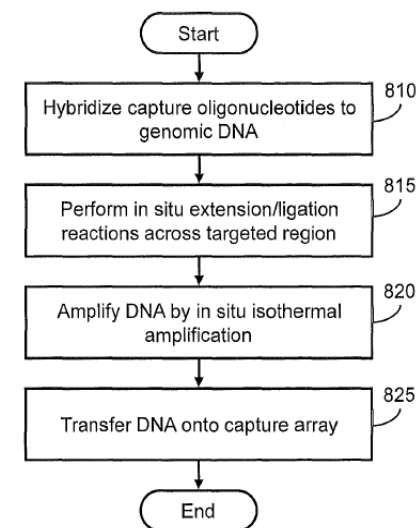


Figure 8

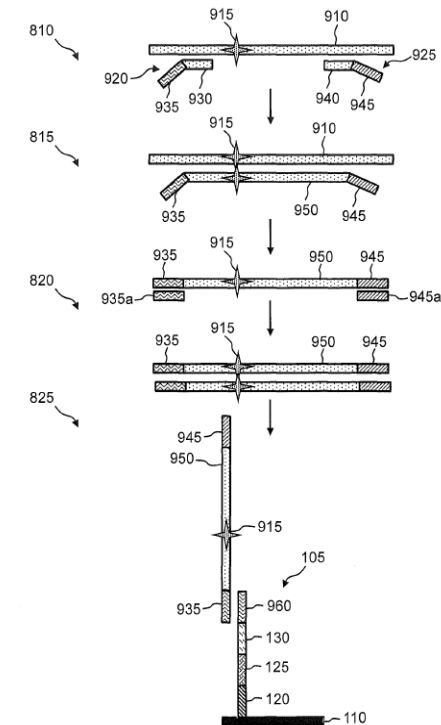


Figure 9

EX1005, Fig. 8-9

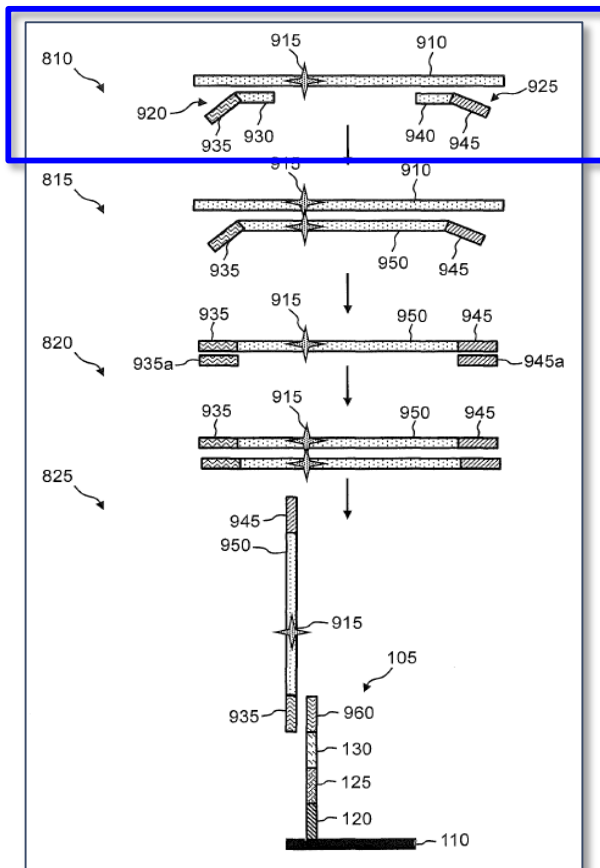
FIG. 8-9's method follows FIG. 2's workflow

So-456's method includes the claimed contacting step

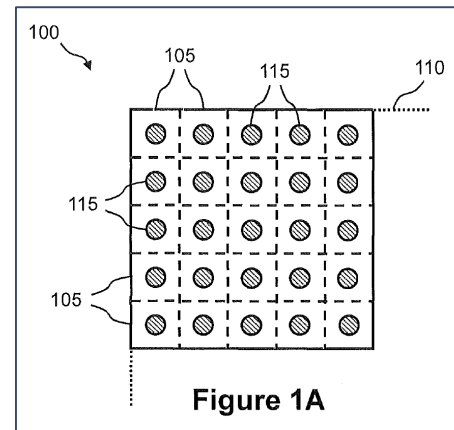
So-456's method includes the claimed contacting step

Claim 16: a) **contacting the tissue sample with a first plurality of nucleic acid probes and a second plurality of nucleic acid probes**, wherein each of the nucleic acid probes in the first plurality of nucleic acid probes comprise a **target binding domain that binds to the at least one target analyte**, wherein each of the nucleic acid probes in the second plurality of nucleic acid probes comprise a **target binding domain that binds to the at least one target analyte**, wherein the tissue sample is treated to facilitate binding of the first plurality of nucleic acid probes and the second plurality of nucleic acid probes to the at least one target analyte;

EX1001, claim 16



EX1005, Fig. 9



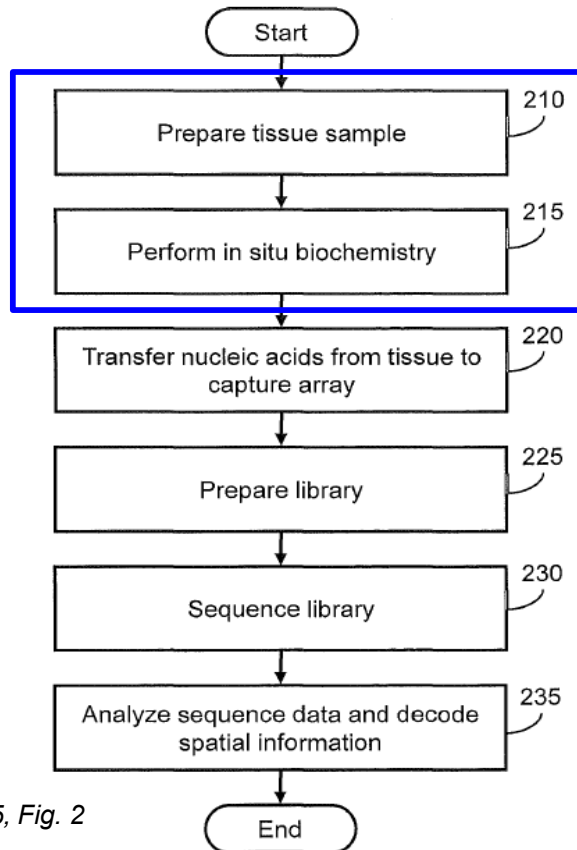
EX1005, Fig. 1A

Nucleic acid probes bind to target analytes in the tissue sample

So-456 expressly teaches treating a tissue sample as claimed

Claim 16: a) **contacting the tissue sample ...**, wherein the **tissue sample is treated to facilitate binding** of the first plurality of **nucleic acid probes** and the second plurality of nucleic acid probes **to the at least one target analyte**;

EX1001, claim 16



EX1005, Fig. 2

FIG. 2

[00152] At a step 210, a tissue sample is prepared for analysis. In some embodiments, the tissue sample is a FFPE tissue sample that is sectioned onto a slide. Other examples include fresh tissue, frozen tissue, etc.

[00153] At a step 215, in situ biochemistry is performed on the tissue section to facilitate subsequent manipulation of a nucleic acid in the sample. In some embodiments, an in situ reverse transcription reaction is used to synthesize cDNA from targeted mRNA in the tissue sample. In some embodiments, an in situ amplification reaction can be used to produce multiple genomic DNA amplicons from targeted genes in the tissue sample. In some embodiments, there is no in situ biochemistry step, and synthesis of cDNA is performed after capture or extraction of the RNA from the tissue.

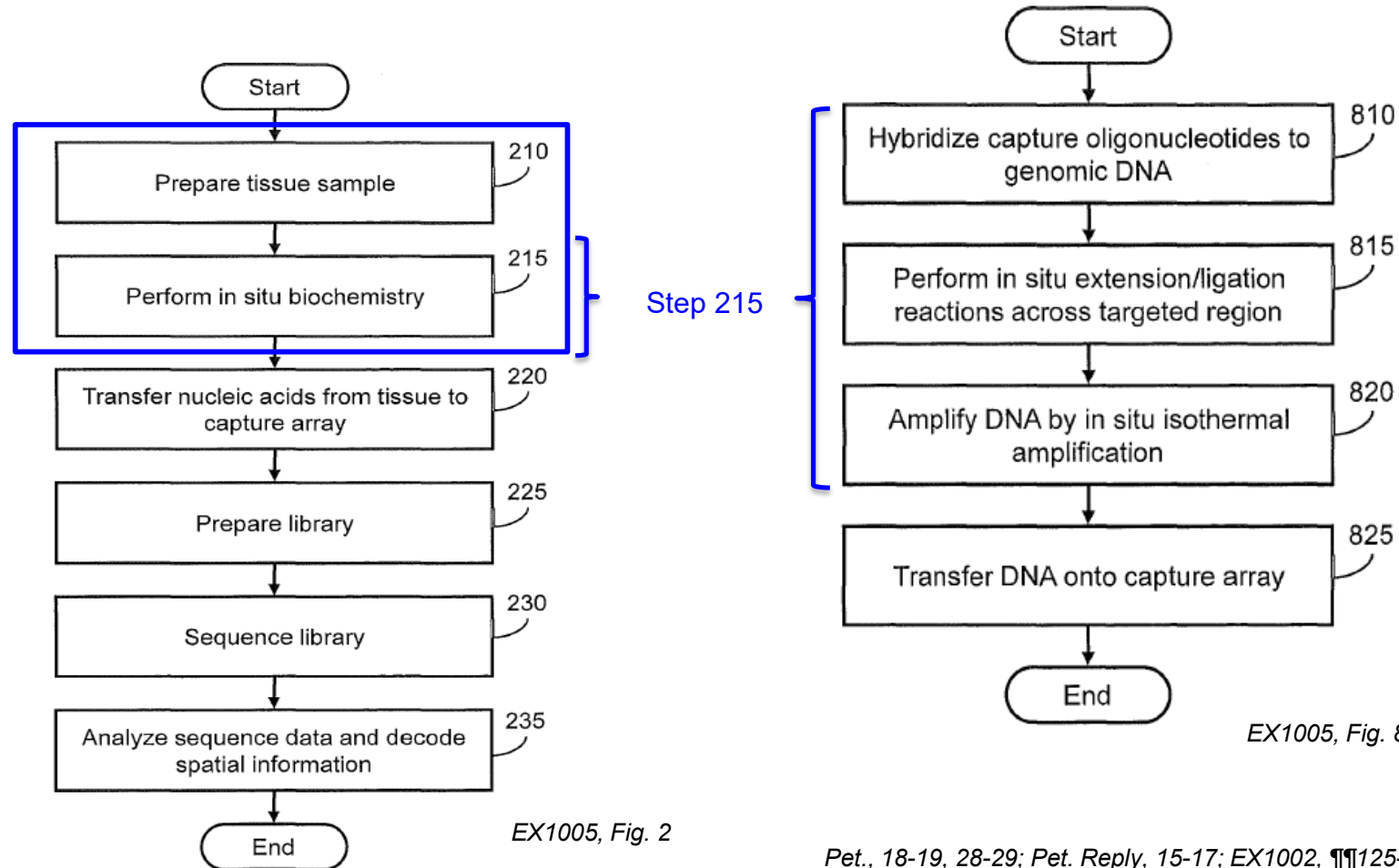
EX1005, ¶¶[00152]-[00153]

Steps 210 and 215 occur **before** the nucleic acid probes bind to the target analytes
Steps 210 and 215 are not limited to the specified “embodiments”

FIG. 2 provides overall workflow of FIG. 8-9's method

Claim 16: a) **contacting the tissue sample . . .**, wherein the **tissue sample is treated to facilitate binding** of the first plurality of **nucleic acid probes** and the second plurality of nucleic acid probes **to the at least one target analyte**;

EX1001, claim 16



Pet., 18-19, 28-29; Pet. Reply, 15-17; EX1002, ¶¶125-127, 164-165; EX1055, ¶¶13-21

Any treatment that facilitates binding of probes to target analytes satisfies the claim

Dr. Spellman:

20. Moreover, the treatment used to facilitate probe binding varies depending on the type of tissue sample, sample's prior treatments, and the objective of the experiment. Appropriate treatments include, depending on the tissue sample, prior treatment, and experiment objective, for example, thawing, sectioning, immobilization, de-paraffinization, de-crosslinking, and/or permeabilization. Dr. Sherman agrees that treatments to facilitate binding of the

to treat a tissue sample.”). Further, the same sample type can be treated in multiple ways.

EX1055, ¶20

Patent Owner misrepresents by alleging that Petitioner “**does not dispute** that the only techniques ... in paragraphs 152-153 have nothing to do facilitating probe hybridization”

Pet. Sur-Reply, 22

Dr. Sherman:

At the time this was added, the Applicant pointed the Examiner to paragraph 266 of the Application, explaining that “there are reagents, for example buffers and other reagents, that are known in the art to be useful in facilitating binding of a protein or a nucleic acid in a sample,” and so it would be “well within the purview of a person of ordinary skill in the art” to treat a tissue sample as recited by the claim. EX1012 at 1560. In my opinion, this is consistent with the state of the art as of the priority date of the '689 Patent.

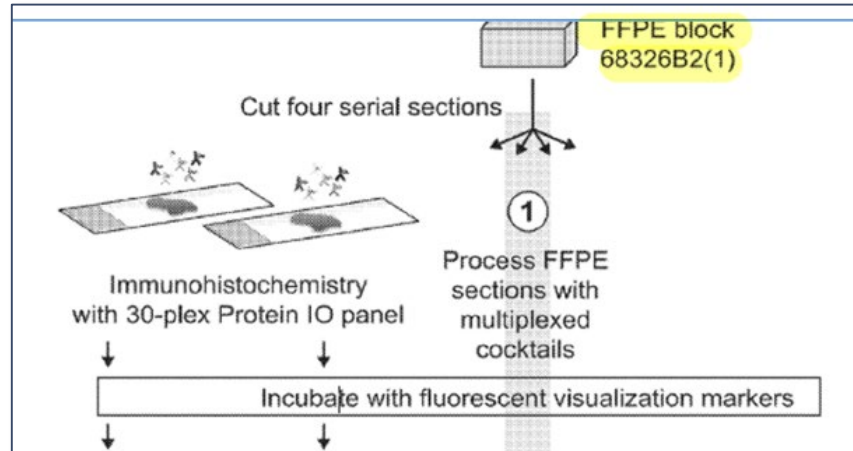
EX2006, ¶48

Treatments to facilitate probe binding were known in the prior art

Pet., 18-19, 28-29; Pet. Reply, 17; EX1002, ¶¶125-127, 164-165; EX1055, ¶¶13-21

So-456 need not disclose the same treatment as the 689 patent's cited disclosure

Patent Owner:



POR, 18

Claim 16:

16. A method for spatially detecting at least one target analyte in a first location and a second location of **a tissue sample** comprising:

a) contacting **the tissue sample** with a first plurality of nucleic acid probes and a second plurality of nucleic acid probes, . . . wherein **the tissue sample is treated** to facilitate binding of the first plurality of nucleic acid probes and the second plurality of nucleic acid probes to the at least one target analyte; . . .

EX1001, claim 16

So-456:

[00152] At a step 210, a **tissue sample is prepared for analysis**. In some embodiments, the tissue sample is a **FFPE tissue sample** that is sectioned onto a slide. Other examples include **fresh tissue, frozen tissue, etc.**

EX1005, ¶[00152]

Neither the challenged claims nor So-456's method is limited to FFPE samples

The Petition also showed that So-456 *inherently* teaches treating a tissue sample as claimed

Petition:

[00153]; EX1002, ¶¶125-127. Further, So-456's Figure 2 depicts performing "in situ biochemistry" on the tissue sample, which facilitates probe binding because Figure 8 depicts "[h]ybridiz[ing] capture oligonucleotides to genomic DNA" (*i.e.*, binding). EX1005, FIGs. 2, 8, 9, ¶¶[00153], [00178], [00182]; EX1002, ¶¶125-127. Thus, So-456's tissue was treated to facilitate probe binding, as claimed. *Id.*

Pet., 18-19

Spellman Original Decl.:

DNA" (*i.e.*, binding). EX1005, FIGs. 2, 8, 9, ¶¶[00153], [00182]. Because the capture oligonucleotides hybridized to the target analytes and were processed according to the subsequent steps, So-456's method treated the tissue sample to facilitate binding of the first and second plurality of capture oligonucleotides (*i.e.*, nucleic acid probes of the claim) to the target DNA molecules (*i.e.*, target analytes).

EX1002, ¶126

See also *Pet. Reply*, 16; EX1055, ¶18

Patent Owner's reliance on *Nidec* is misplaced

In *Nidec*, the prior art reference did not disclose the claim element expressly *or inherently*



“Because Kusaka does not disclose a signal in the rotating frame of reference, **it does not disclose an IQdr Demand [the claim element].**”

“The Board's misapplication of *Kennametal* improperly **assumed disclosure of a claim element.**”

“*Kennametal* addresses whether the disclosure of a limited number of combination possibilities discloses one of the possible combinations.”

Nidec Motor Corp. v. Zhongshan Broad Ocean Motor Co. Ltd., 851 F.3d 1270, 1274 (Fed. Cir. 2017)

So-456's method includes the claimed ligation step

The challenged claims include ligating *non*-adjacent probes by gap extension and nick repair

Claim 16:

b) forming ligated probes by **ligating** each of nucleic acid probes from the first plurality of nucleic acid probes and each of nucleic acid probes from the second plurality of nucleic acid probes that are **bound to each of identical molecules from the at least one target analyte**;

EX1001, 147:54-148:11

689 patent specification:

Following hybridization of the first and the second nucleic acid probe to the identifier oligonucleotide, a short probe hybridization method can further comprise: (5) in the aspect in which the first and the second nucleic acid probe hybridize to the identifier oligonucleotide such that the first and the second nucleic acid probes are adjacent and are not overlapping, ligating the first and the second nucleic acid probes together, for example, by performing a nick repair reaction. Alternatively, in the aspect in which the first and the second nucleic acid probe hybridize to the identifier oligonucleotide such that the first and the second nucleic acid probes are not adjacent and are not overlapping, the method comprises ligating the first and the second nucleic acid probes together, for example, by performing a gap extension reaction and a nick repair reaction, such that the first and the second nucleic acid probes are ligated together.

EX1001, 25:66-26:6; see also 6:66-7:4, 7:34-40, 9:48-54, 10:19-24, 30:59-65, 68:66-69:3, 70:40-44

So-456's method includes the claimed ligation step

So-456:

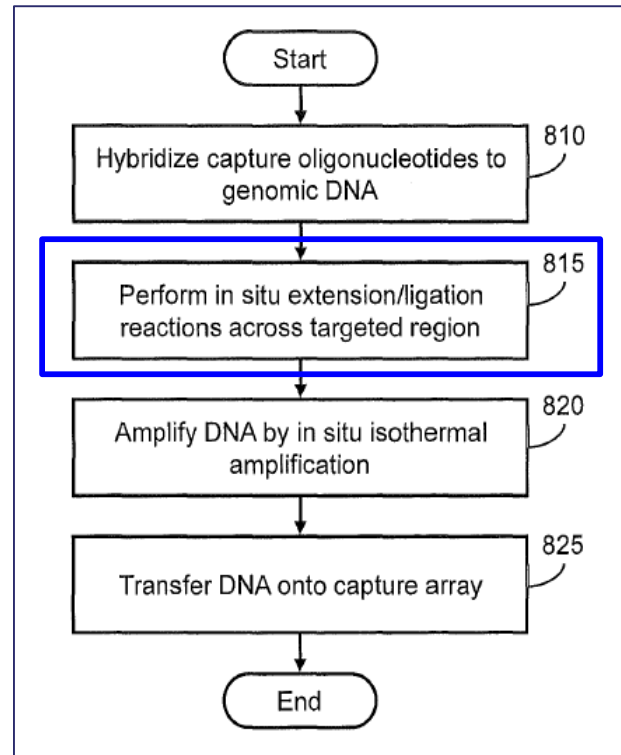


FIG. 8

EX1005, Fig. 8

So-456:

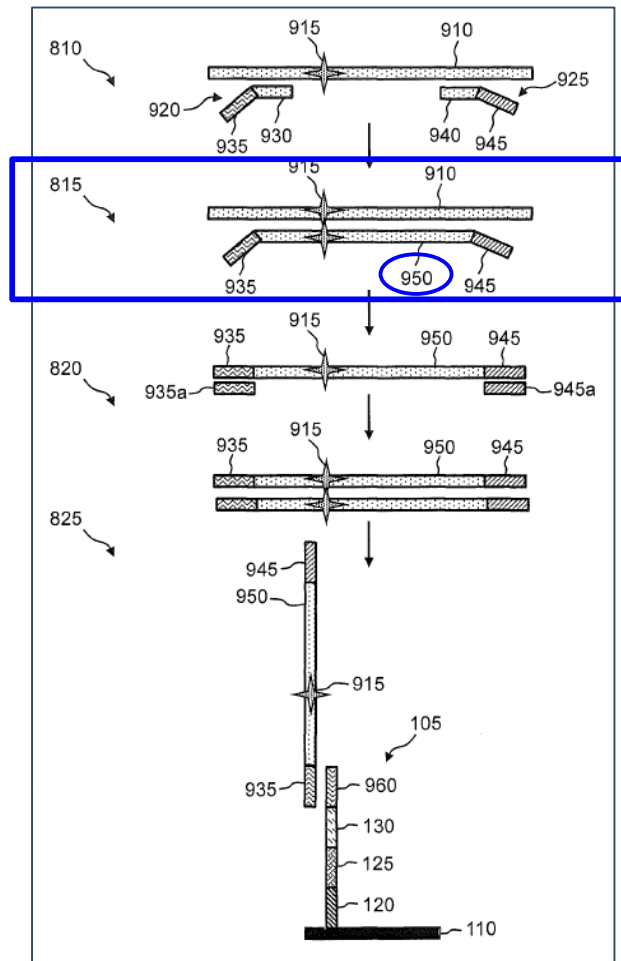


FIG. 9 EX1005, Fig. 9

So-456:

[00182] Figure 9 illustrates an embodiment of the steps of a method 800 of Figure 8....
At step 815, an extension/ligation reaction is performed in situ between the flanking capture oligonucleotides 920 and 925 across the region of interest. **A DNA molecule 950 that is formed in the extension/ligation reaction** comprises universal capture region 935 and SBS primer region 945.

EX1005, ¶[00182]

So-456's extension/ligation = 689 patent's gap extension/ligation:

- Same extension enzyme (DNA polymerase)
- Same ligation enzyme (DNA ligase)
- Same reaction

Pet. Reply, 18; EX1055, ¶33; EX1056, 35:11-17, 55:9-57:3

Pet., 19-20; Pet. Reply, 17-19; EX1002, ¶¶128-130; EX1055, ¶¶22-34

The intent and size of So-456's extension reaction are irrelevant to the challenged claims

Patent Owner:

reaction, this is incorrect. As Dr. Sherman explains, the “gap extension reaction” merely exists in the case where the probes are not close enough to each other to successfully ligate. EX2006 ¶¶ 71-72. Unlike So-456's extension/ligation, it is not meant to capture an amplicon to collect meaningful data, and a skilled artisan would not equate the two. *Id.* Accordingly, So-456 does not disclose the ligation

POR, 37

As Dr. Sherman explained at deposition, “visually, [So-456's] Figure 9, you can see that it's a large gap...it's a region that needs to be extended through a evidently large region.” EX1056 at 77:24-78:1. That is because the purpose of Figure 9's large gap is for an extension to capture a mutation in the target analyte. EX2006 ¶ 70. In contrast, the '689 patent discloses a small gap extension before ligation where the nucleic acid probes are not adjacent and not overlapping. EX1056 at 78:2-7. No mutations are incorporated into the ligated probe.

PO Sur-Reply, 27

No user intent or gap size requirement in the claims

No evidence in the 689 patent that the gap between the non-adjacent probes is “small”

So-456's FIG. 9 illustrates an example and is not an accurate depiction of the gap size

So-456's method includes the claimed collecting steps

Petition is clear: So-456's capture of the original "ligated probes" meets the claimed collecting steps

Petition:

The capture site comprises immobilized array probes ("capture probes" 115) containing a spatial barcode ("spatial address region" 130) and a "capture region" (135 or 960) that hybridizes a complementary region on the ligated probes ("universal capture region" 935). EX1005, ¶¶[00141], [00182]-[00183]; EX1002, ¶¶135-137. These details are depicted in Dr. Spellman's annotations of So-456's Figures 9 and 1A:

¶¶[00141], [00183], FIGs. 1A, 9; EX1002, ¶¶136-137. Thus, So-456's method collects *whole* ligated primary probes from the *whole* tissue sample at the same time, as claimed according to NanoString's construction. EX1002, ¶¶136-137; EX1008, 19-20; EX1020, 53-54.

Pet., 20-22; see also *id.* 29

Spellman Original Decl.:

137. Accordingly, So-456's method includes capturing *whole* ligated probes (formed by ligating the primary probe pairs) that diffuse from and are captured across the *whole* tissue sample, all at the same time. Thus, in my opinion, So-456's method includes claim 16's collecting steps under NanoString's interpretation.

169. As I explain for claim 16, So-456's method includes capturing *whole* ligated probes (formed by ligating the primary probe pairs) that diffuse from and are captured across the *whole* tissue sample, all at the same time. Accordingly, for these reasons (and those explained in detail for claim 16), So-456's method includes collecting *whole* ligated probes that were previously formed in the ligation step under conditions that release them from the *whole* tissue sample at the same time. Thus, in my opinion, So-456's method includes claim 1's collecting steps under NanoString's interpretation.

EX1002, ¶¶137, 169

The Petition showed that So-456's method collects the original ligated probes (950) and their amplicons (950)

Claim 16: c) [and (d)] **collecting the ligated probes, or portions thereof**, bound to each of the identical molecules from the at least one target analyte in a **first location** [(d): **second location**] of the tissue sample under conditions that release the ligated probes, or portions thereof, **from the first location** [(d): **second location**] of the tissue sample;

EX1001, claim 16

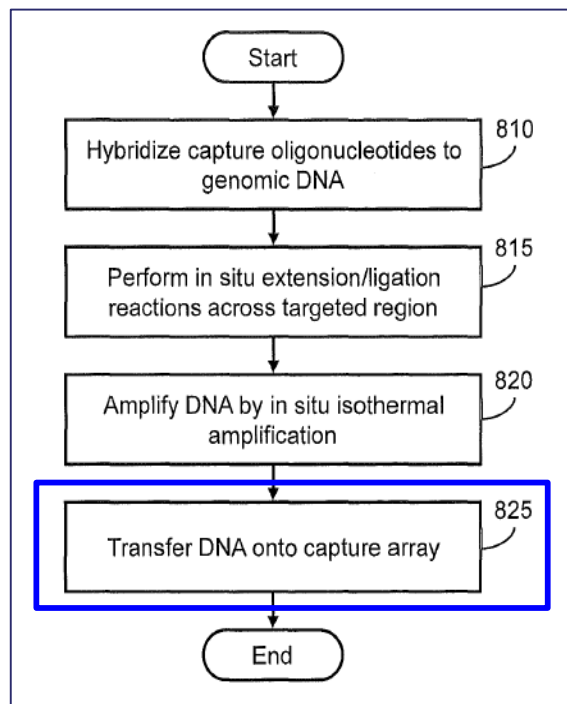


FIG. 8 EX1005, Fig. 8

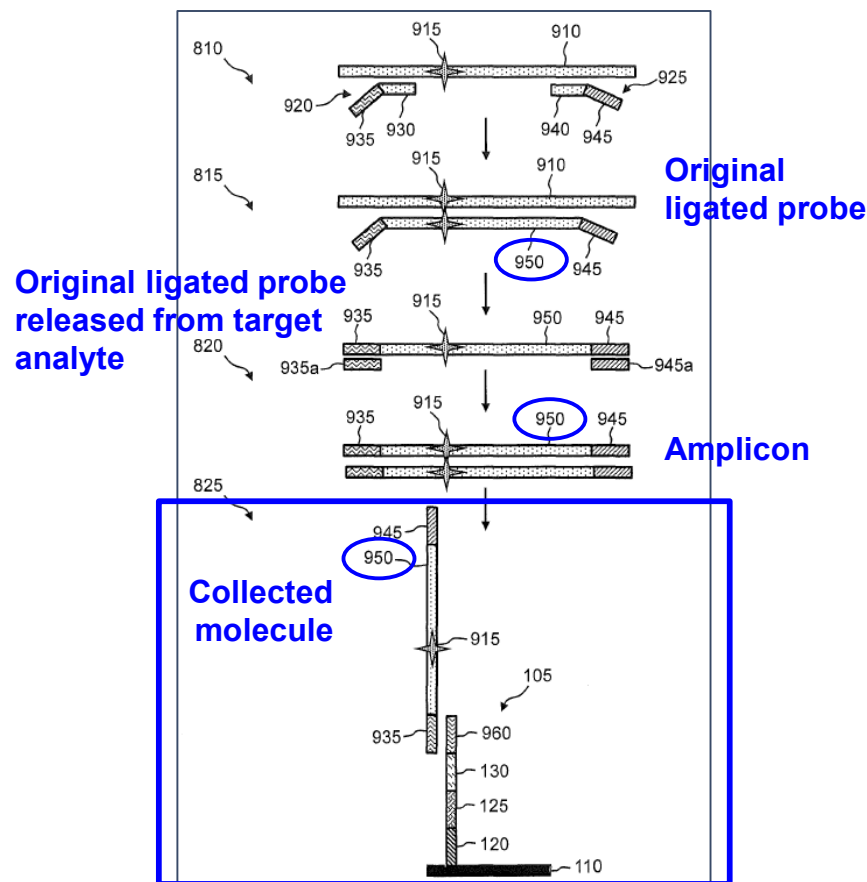


FIG. 9

Pet., 20-22, 29; EX1002, ¶¶131-137; see also Pet. Reply, 19-21; EX1055, ¶¶35-41

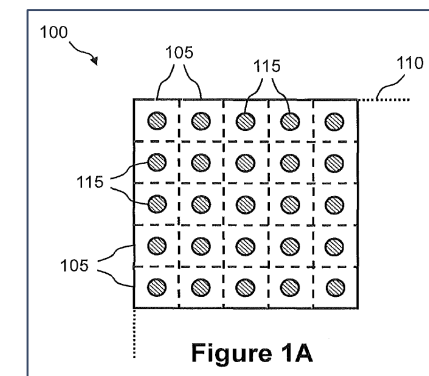


Figure 1A

EX1005, Fig. 1A

EX1005, Fig. 9

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Patent Owner admits that So-456's original ligated probes and collected molecules contain the same segments

Dr. Sherman:

Q. All right, Dr. Sherman, just before the break we were looking at Figure 9 in the '456 reference, so I'd like to continue discussing that figure with you.

Step 825 at the bottom of Figure 9 depicts molecule 950 encapsulated on a capture probe on an array; is that correct?

A. That's what I see.

Q. And this molecule 950 comprises a segment 935; is that right?

A. That's right.

Q. And also comprises segment 945; is that correct?

A. Right.

Q. And segment 935 is also present in the original molecule 950 formed in step 815. Correct?

A. 935 is found in the original.

Q. And same question for 945, it was also present in the original molecule 950. Correct?

A. Right. The -- but this is a copy of that.

Q. All right. Let's look at the capture probe part of this figure at the bottom of Figure 9. The capture probe here has four labeled segments. Do you see that?

Original ligated probes



Segment 935?



Segment 945?

Collected molecules

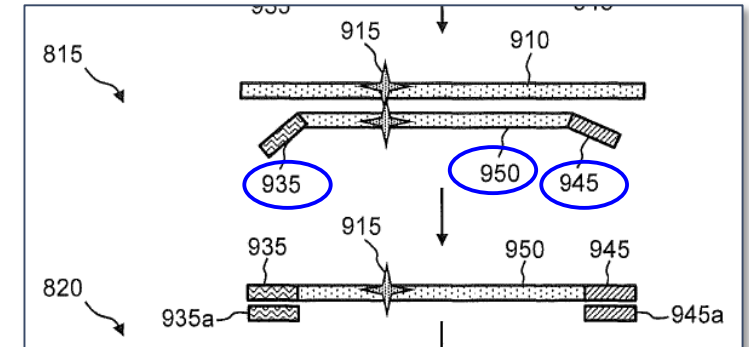


Segment 935?

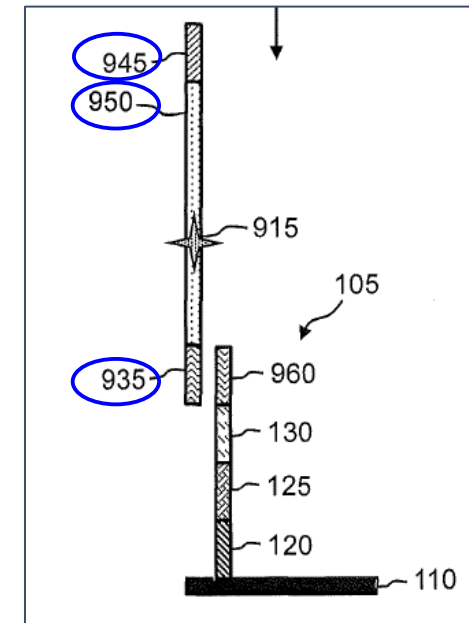


Segment 945?

So-456:



EX1005, Fig. 9



EX1005, Fig. 9

EX1056, 44:13-45:5

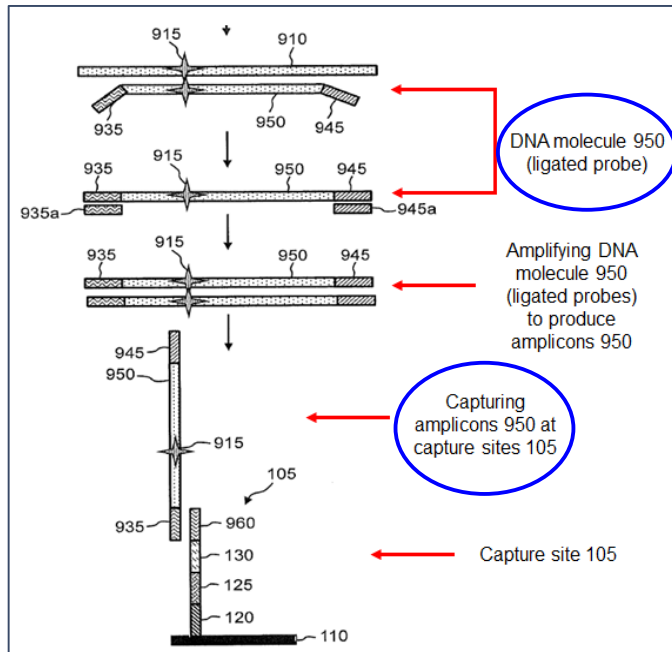
DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Pet. Reply, 20-21; EX1055, ¶40

Petitioner interchangeably used “ligated probe” and “amplicon”

Petitioner:

The capture site comprises immobilized array probes (“capture probes” 115) containing a spatial barcode (“spatial address region” 130) and a “capture region” (135 or 960) that hybridizes a complementary region on the ligated probes (“universal capture region” 935). EX1005, ¶¶[00141], [00182]-[00183]; EX1002, ¶¶135-137. These details are depicted in Dr. Spellman’s annotations of So-456’s Figures 9 and 1A:



Pet., 20-21; EX1002, ¶135 (p. 72)

Dr. Spellman:

135. These amplicons (950) (i.e., ligated probes of the claim) are “captured onto the capture site 105” of a spatially barcoded array, such as an array in Figure 1A. EX1005, ¶¶[00182], [00180], [00181], FIGs. 1, 8, 9. The annotated excerpts of Figures 9 and 1A below depict these steps in So-456’s method.

EX1002, ¶135 (p. 72)

Q Do you see an annotated Figure 9 at Paragraph 135?

...

Q Sitting here today, is there any reason for you to change any of these annotations?

A Using -- the **words on Page 72 are my own, not the language of the '456 [sic] specification**, which uses a very specific definition. And the language used on Page 72 is **my own language, rather than the language consistent with '456 [sic]**, which would, in that context, **the first extension product copied from 910 [i.e., original ligated probe] would be an amplicon.**

Q So at this step of capturing Amplicons 950 at capture site 105, are you saying that copies of the original ligated probes generated at Step 820 is captured?

A The -- my declaration in **Paragraph 135 explicitly states**, These **Amplicons 950, i.e., ligated probes of the claim, are captured** onto the capture site 105." My declaration **equates the first amplicon copy 950 synthesized by the extension ligation reaction [i.e., original ligated probe] with any derived copies of 950 synthesized at Step 820.**

Patent Owner misrepresents the “drastically low likelihood” of capturing the original ligated probes

Patent Owner:

show that the “original ligated probes” are captured. Dr. Spellman’s testimony confirms the drastically low likelihood of capturing the “original ligated probes.”

PO Sur-Reply, 20

So-456:

The number of copies of a nucleic acid template can be **at least 1**, 10, 100, 200, 500, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000 and 10,000 copies, and can be varied depending on the particular application.

EX1005, ¶¶00122]

Dr. Spellman:

Q Dr. Spellman, are you saying the maximum number of copies is 10,000?

MS. KHANDURI: Objection. Form.

A My apologies. It says **at least one** and as many as at least 10,000.

Q So do you agree that there can be at least 10,000 copies of a template from an amplification reaction?

MS. KHANDURI: Objection. Form.

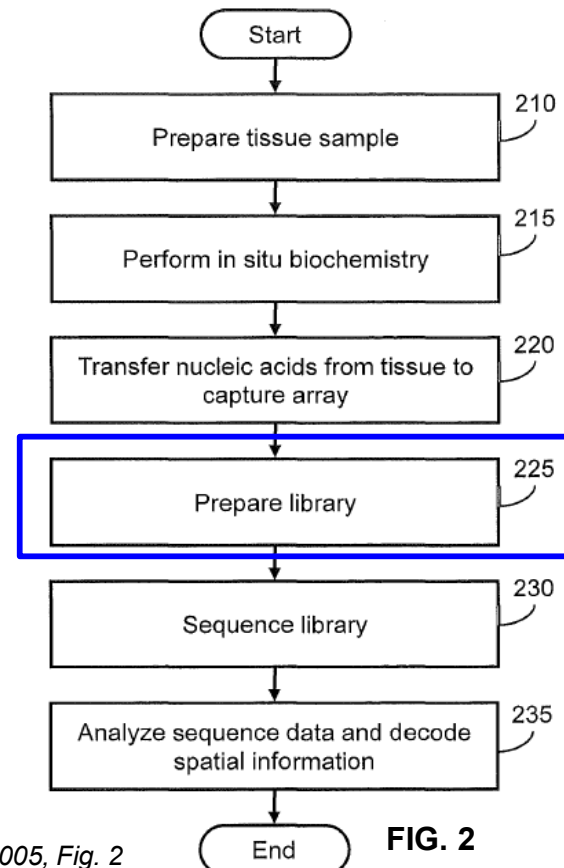
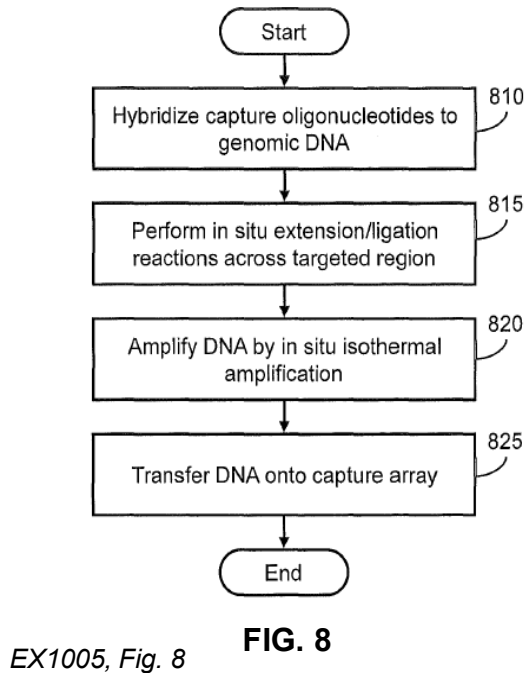
A Paragraph 122 instructs a POSA to modify their replication technology to influence amplification yield and control the number of copies as they see fit from as -- **at one copy** to as many as 10,000 or more copies.

EX2053, 36:1-14

So-456's method includes the claimed extension steps

So-456's method includes the claimed extension steps

Claim 16: e) [and (f)] **performing an extension reaction that incorporates at least one nucleic acid sequence that identifies the first [and second] location of the tissue sample into each of the ligated probes, or portions thereof, collected in step (c) [and (d)], thereby forming a first [(f): second] plurality of extension products that comprise the ligated probes, or portions thereof, collected in step (c) [and (d)] and the at least one nucleic acid sequence that identifies the first [(f): second] location of the tissue sample**



So-456's method includes the claimed extension steps during library preparation for sequencing (Step 225)

FIG 8-9's method requires tagging the collected ligated probes with a spatial barcode during library preparation

So-456:

[0089] The present disclosure is based, in part, on the realization that information related to the spatial origin of a nucleic acid in a tissue sample can be encoded in the nucleic acid in the process of preparing the nucleic acid for sequencing. For example, nucleic acids from a tissue

EX1005, ¶[0089]

So-456's methods are **based on encoding a spatial barcode** into the analyzed molecules **during library preparation**

Petition:

However, So-456's ligated probes (950) captured on the array in the previous step are **not yet tagged with a spatial barcode** and do not include functional sequences (*i.e.*, a pair of SBS primer-binding sites) necessary for performing sequencing-by-synthesis (SBS) in subsequent steps. EX1002, ¶142. Therefore, **during sequencing library preparation** (Step 225)—the only step remaining before the sequencing step (Step 230)—**So-456's method necessarily generates molecules containing a spatial barcode and sequencing primer-binding sites**, such that the molecules are ready for sequencing, analysis, and decoding in the subsequent steps (Steps 230, 235). EX1005, ¶¶[00156]-[00158], FIG. 2; EX1002, ¶142.

Pet., 24

So-456's FIG. 8-9 method:

- The collected ligated probes **not yet tagged** with a spatial barcode
- The collected ligated probes **must be tagged** with a spatial barcode **during library preparation before they are released** from the capture array in order to retain the spatial information

Pet., 23-27, 30-31; EX1002, ¶¶138-152, 170-184

FIG. 8-9's method uses FIG. 1's capture probes that contain a spatial barcode

So-456:

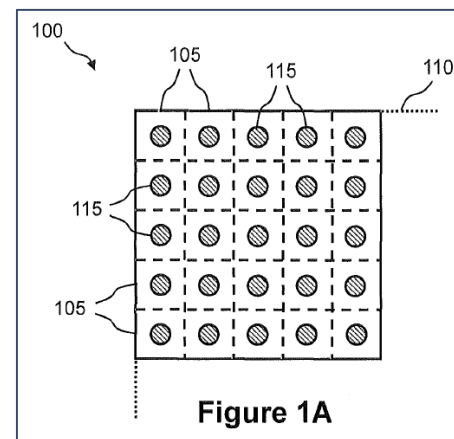
[00177] Figure 8 illustrates a flow diagram of an embodiment of a method 800 of capturing DNA amplicons onto an array (e.g., capture array 100 of Figure 1A), wherein the DNA amplicons are generated by in situ amplification of target nucleic acid. In this embodiment, the

[00182] Figure 9 illustrates an embodiment of the steps of a method 800 of Figure 8.

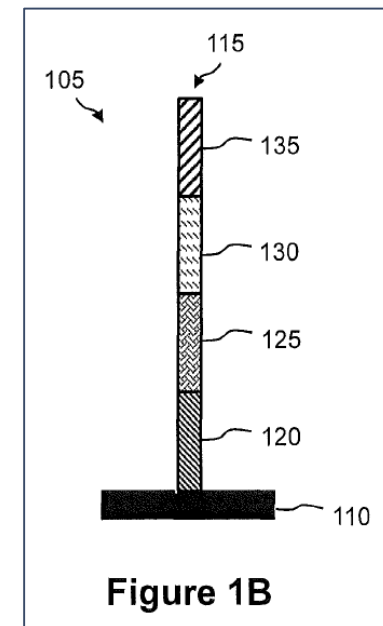
[00141] Figure 1A illustrates a plan view of an exemplary embodiment of a capture array 100 for capture of nucleic acid in a tissue sample. Capture array 100 comprises an

each of the capture sites 105 of capture array 100, as shown in Figure 1B. Namely, Figure 1B illustrates a side view of one capture site 105 of capture array 100, wherein the one capture site 105 comprises at least one capture probe 115 for capture of nucleic acid in a tissue sample.

EX1005, ¶¶[00177], [00182], [00141]



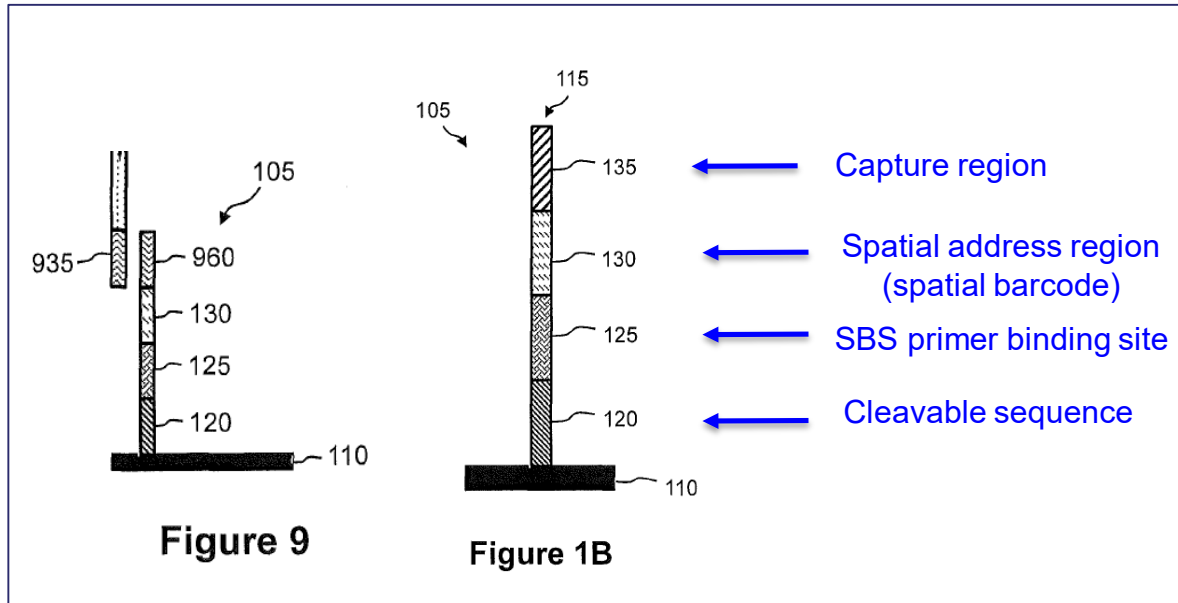
EX1005, Fig. 1A



EX1005, Fig. 1B

Segment 130 in FIG. 8-9's capture probe is a spatial barcode

So-456:



EX1005, Fig. 9, Fig. 1

So-456:

[00141] . . . **Figure 1B** shows a capture probe **115** immobilized on the surface of solid support **110**. In this embodiment, a single capture probe **115** is shown, but any number of capture probes **115** can be immobilized on solid support **110** at each capture site **105**. **Capture probe 115** may optionally comprise a **cleavable sequence in a cleavable region 120**, an **SBS primer sequence in a SBS primer binding site 125**, a **spatial address sequence in a spatial address region 130**, and a **capture sequence in a capture region 135**.

EX1005, ¶[00141]

FIG. 8-9's and FIG. 1's capture probes contain the same segments

FIG. 9's identification of Segment 130 as a SBS primer region is a clear typographical error

So-456:

Figure 9 illustrates an embodiment of the steps of a method 800 of Figure 8. . . . In this example, capture probe 115 on the capture site 105 comprises a capture region 960 that is complementary to universal capture region 935; SBS primer region 130 is SBS3.

EX1005, ¶ [00182]

Dr. Spellman:

55. My first declaration also correctly identified segment 130 in Figure 8-9's capture probes as a spatial barcode. EX1002, ¶¶99, 104, 143, 175. So-456's paragraph 141 identifies segment 130 as a "spatial address region," i.e., a spatial barcode. EX1005, ¶ [00141]. So-456 also repeatedly identifies segment 130 as a spatial barcode throughout its disclosure, except in Figures 6 and 8-9. EX1005, ¶¶ [00141], [00145], [00146], [00173], [00189], [00201], [00202], [00213], [00214], FIGs. 1B, 1C, 3, 4, 6, 7, 8, 9, 11, 13A, 13B, 15B, 15C.

EX1055., ¶55

For example, So-456:

Capture probe 115 may optionally comprise a cleavable sequence in a cleavable region 120, an SBS primer sequence in a SBS primer binding site 125, a spatial address sequence in a spatial address region 130, and a capture sequence in a capture region 135.

Universal adaptor oligonucleotide 160 is essentially the same as capture probe 115 of Figure 1B except that capture region 135 is omitted, i.e., universal adaptor oligonucleotide 160 comprises only cleavable region 120, SBS primer region 125, and spatial address region 130.

When universal capture region 630 is used as a primer, spatial address region 130 and SBS primer region 125 are copied.

At step 1025, concatamer 1155 is captured onto the capture site 105 that comprises cleavable region 120, SBS sequencing primer region 125 (e.g., SBS3), spatial address region 130, and capture region 135 (e.g., a universal capture sequence).

EX1005, ¶¶ [00141], [00145], [00173], [00189]

So-456's FIG. 6 disclosure includes a similar typographical error for Segment 130

So-456:

Figure 6 illustrates an embodiment of the steps of a method 500 of Figure 5. . . . In some embodiments, **capture probe 115 on the capture site 105** comprises a capture region 660 that is complementary to universal capture region 630; **SBS sequencing primer 130** is SBS3. DNA molecule 655 is captured onto the capture site 105 by hybridization of universal capture region 630 to capture region 660.

In this example, both capture region 660 and universal capture region 630 can also function as primers for an extension reaction. When capture region 660 is used as a primer, mutation 615 in DNA molecule 655 is copied. When universal capture region 630 is used as a primer, **spatial address region 130** and **SBS primer region 125** are copied. Both extension products can be used for downstream library generation.

EX1005., ¶¶172, 173

Dr. Spellman:

¶¶[00172]-[00173], [00182]-[00183]. Figure 6's disclosure identifies **segment 130** as *both* an "SBS sequencing primer" and a "spatial address region," while at the same time it identifies **segment 125** also as an "SBS primer region." EX1005, ¶¶[00172]-[00173]. Given that So-456 repeatedly describes segment 130 as a **spatial barcode** (see, e.g., EX1005, ¶¶[00141], [00145], [00146], [00172], [00183], [00189], [00201], [00202], [00213], [00214], FIGs. 1B, 1C, 3, 4, 6, 7, 8, 9, 11, 13A, 13B, 15B, 15C), a POSA would readily recognize that **segment 130** should be the spatial barcode and **segment 125** should be the SBS primer region in Figure 6's capture probes. A POSA would also readily recognize that Figure 9's disclosure repeats Figure 6 disclosure's error by identifying segment 130 as an SBS primer region. Thus, a POSA would readily know that segment 130 in Figure 9's (and Figure 6's) capture probe is a spatial barcode, and not an SBS primer region.

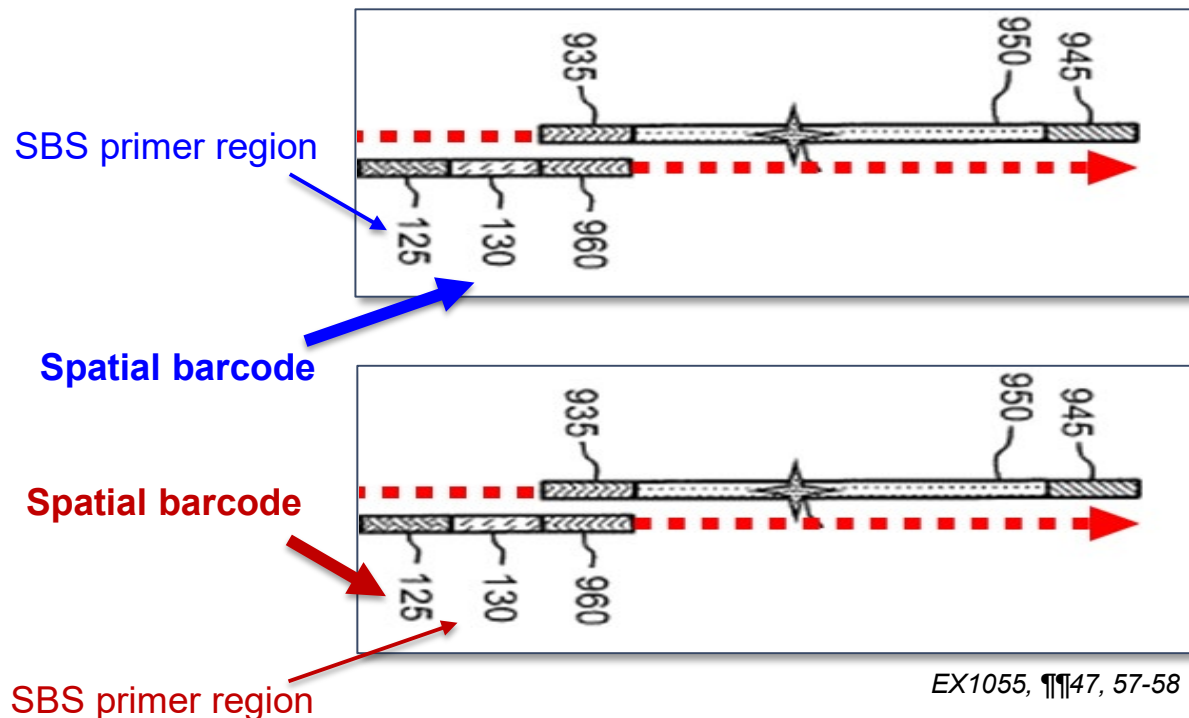
EX1055, ¶56

Locating the spatial barcode 5' of the SBS primer would defeat the purpose of So-456's methods

Dr. Spellman:

“A **spatial barcode must be downstream (3') of the SBS primer region (125)** ... Because segment 130 is downstream (3') of segment 125, they must be the spatial barcode and SBS primer region, respectively.”

EX1055, ¶157



EX1055, ¶¶47, 57-58

If segment 125 is a spatial barcode, **it would not be part of the sequenced molecule:**

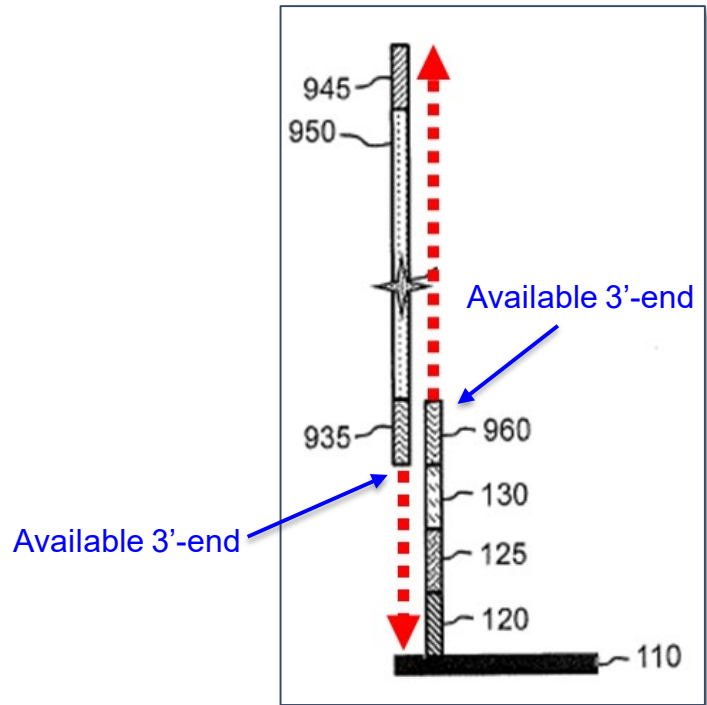
Dr. Sherman:

Q. Okay. So segment 125 would not be part of that sequencing; is that correct?

A. **That's correct.**

EX1056, 46:19-21

So-456's method includes bidirectional extension that incorporates a spatial barcode into the collected ligated probes



EX1002, ¶182

So-456:

[00109] As used herein, the term "immobilized" when used in reference to a nucleic acid is intended to mean direct or indirect attachment to a solid support. . . . Oligonucleotides to be used as **capture primers** or **amplification primers** can be immobilized such that a **3'-end is available for enzymatic extension** and at least a portion of the sequence is capable of hybridizing to a complementary sequence.

EX1005, ¶[00109]

Dr. Spellman:

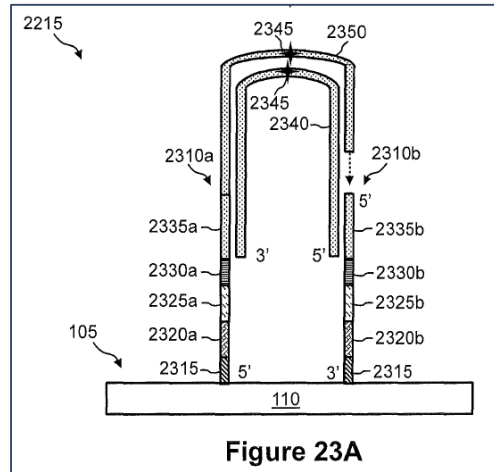
“So-456 does not disclose any blocking or other modifications to the 3'-end of the **captured oligonucleotides** that are extended in its methods, allowing extension of both the 3'-end of the captured amplicons and 3'-end of the capture probes in the method of Figures 8-9. EX1005, ¶¶[00109], [00182]-[00183], FIGs. 8, 9. This is because **extension enzymes, such as DNA polymerase, automatically extend all available 3'-ends**. Thus, due to the nature of the extension enzymes and the presence of unblocked and unmodified 3'-ends, **So-456's extension reaction necessarily proceeds in both directions**—extending the 3'-end of the capture probe in one direction and the 3'-end of the captured ligated probe in the other direction. EX1005, ¶¶[00109], [00182]-[00183], FIG. 9.”

EX1002, ¶181

Pet., 24-27, 30-31; Pet. Reply, 21-22, 25-27; EX1002, ¶¶141-152, 170-184; EX1055, ¶¶42-48, 60-69

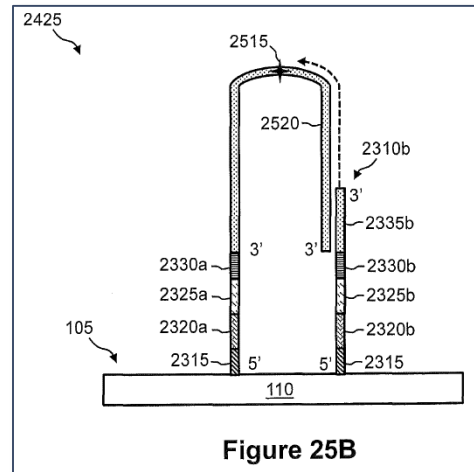
So-456's other embodiments depicting a unidirectional arrow for an extension reaction are irrelevant

So-456:



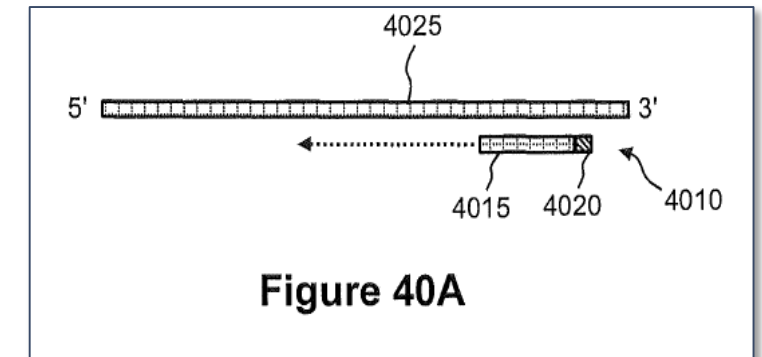
EX1005, Fig. 23A

So-456:



EX1005, Fig. 25B

So-456:



EX1005, Fig. 40A

Dr. Spellman:

“Dr. Sherman’s cited reactions in Figures 22-23A (Step 2215), 24-25A (Step 2415), 28-29A (Step 2815), and 38-39 (Step 3810) are **designed to synthesize from an mRNA template a “first strand cDNA”** for use in the subsequent steps.”

EX1055, ¶62

Dr. Spellman:

“Dr. Sherman’s cited reactions in Figures 24-25B (Step 2425), 28-29A (Step 2820), and 38-39 (Step 3820) are **designed to synthesize a “second strand cDNA” for use in the subsequent steps.**”

EX1055, ¶64

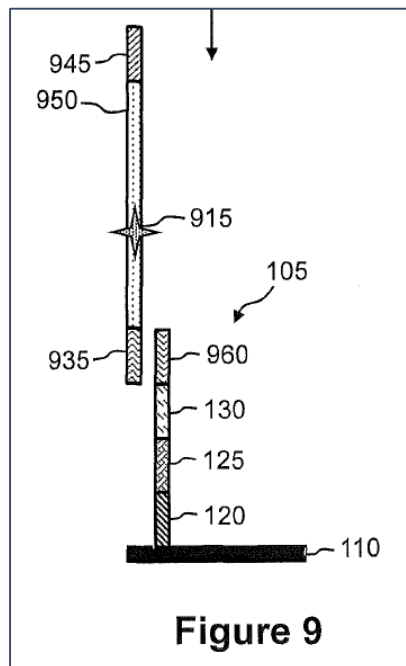
Dr. Spellman:

“[I]n Figure 40 ... The **only 3'-end with an available corresponding template is that of the capture probe** and is thus the only 3'-end that can be extended (shown by the arrow).”

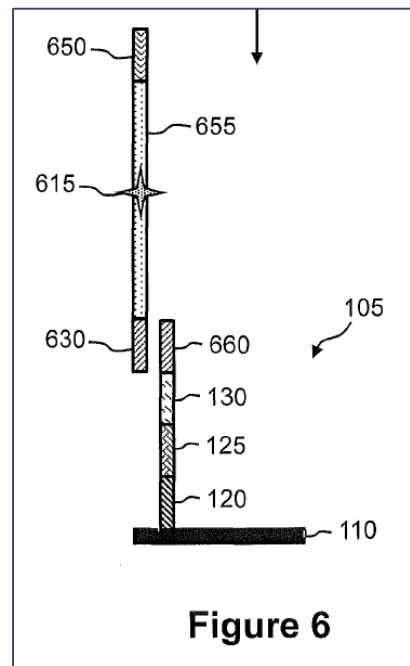
EX1055, ¶65

So-456's methods designed for default bidirectional extension do not depict extension arrows

So-456:



EX1005, Fig. 9



EX1005, Fig. 6

So-456 (Figure 6):

[00173] In this example, both capture region 660 and universal capture region 630 can also function as primers for an extension reaction. When capture region 660 is used as a primer, mutation 615 in DNA molecule 655 is copied. When universal capture region 630 is used as a primer, spatial address region 130 and SBS primer region 125 are copied. Both extension products can be used for downstream library generation.

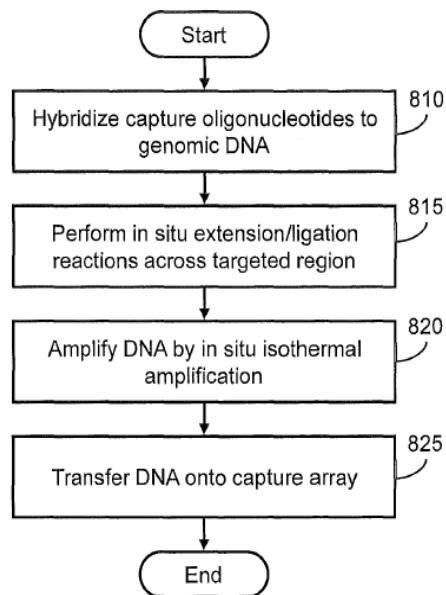
EX1005, ¶[00173]

So-456's method includes the claimed sequencing step

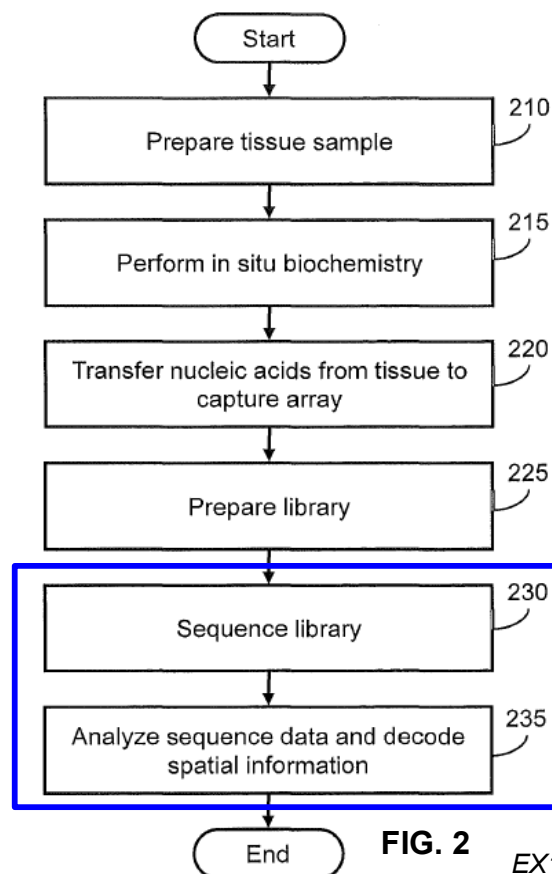
So-456's method includes the claimed sequencing step

Claim 16: g) identifying the first plurality of extension products and the second plurality of extension products by **sequencing the first plurality of extension products and the second plurality of extension products**, thereby spatially detecting the at least one target analyte in the first location of the tissue sample and the second location of the tissue sample

So-456:



EX1005, FIG. 8



EX1005, FIG. 2

So-456:

[00157] At a **step 230**, the library is sequenced. Sequencing may be accomplished using any sequencing technique. Examples of suitable sequencers include those available from, or being developed by, Illumina, Inc., F. Hoffmann-La Roche AG, Life Technologies, Inc., Beckman Coulter, Inc., Pacific Biosciences, Inc., Oxford Nanopore, Inc., and/or their affiliates.

[00158] At a **step 235**, the sequence data is **analyzed** (e.g., mutations and/or variant calling) and the **spatial information is decoded**. The spatial information can be used **to provide information as to the location of the nucleic acid in the tissue section**.

EX1005, ¶¶[00182]

Ground 2: Claims 1-30 would have been obvious over So-456

Ground 3: Claims 9-10 and 24-25 would have been obvious over So-456 and Stahl

The Board agreed that high skill level would have assisted in bridging any gaps between So-456 and the challenged claims



“After reviewing the record of evidence, we find **Petitioner’s case for obviousness is stronger** than its case for anticipation **from a technical perspective due to the high level of skill in the art that could have assisted an ordinary artisan in understanding how to bridge the gaps** between one or more disclosures of So-456 and the challenged claims.”

DOI, 30

There is no dispute that the claimed contacting step would have been obvious over So-456



“[T]o the extent So-456 did not disclose “wherein the tissue sample is treated to facilitate binding of the nucleic acid probes to the target analyte” as recited in claims 1 and 16, **we agree with Petitioner that an ordinarily skilled artisan would have known how to prepare tissue for in situ histochemistry.**”

DOI, 30-31

Patent Owner (487 Appl file history):

of a protein and/or a nucleic acid in a sample. That is, Applicant submits that **it would be well within the purview of a person of ordinary skill in the art** (i.e. a person with at least a PhD in biology) **to treat a tissue sample** such that at least one probe can bind to at least one target analyte in at least one cell in a tissue sample, as such a step is **a routine laboratory procedure** that is commonly performed in both research and industrial labs within the field of the present disclosure.

EX1012, 1560; see also EX2006, ¶48; POR, 19

Dr. Sherman:

Q. In **what routine ways** would a POSA have **treated** an FFPE tissue sample in order to facilitate binding of nucleic acid probes to a target analyte in that tissue sample?

A. **They would have known or there would have been literature on, you know, the basics**, how you create the sample, what sort of buffers you use, what sort of -- you know, how to maintain that sample in a form that would be stable and represent what you're interested in eventually investigating.

EX1056, 50:6-52:2

There is no dispute that the ligation step is a basic skill known in the art

Patent Owner:

“In other words, **ligation of probes is yet another skill that was fundamental and well-known in the art**, such that a POSA would not even need detailed teaching regarding this technique in the '487 application. EX2006 ¶¶ 118-120.”

POR, 56

Dr. Sherman:

“**Ligation is a basic skill well within the competency of a POSA**. I agree with Dr. Spellman that **ligation is a simple skill and well within the basic competencies of a POSA**. *Id.* I also agree with Dr. Spellman that “a person of ordinary skill in the art would be familiar with ligation as a broad concept, extremely broad, and how and when it can be utilized effectively” and that a “POSA has a set of competencies, things they know how to do—ligation being a common one, for example.”

EX2006, ¶119

The claimed collecting steps would have been obvious over So-456

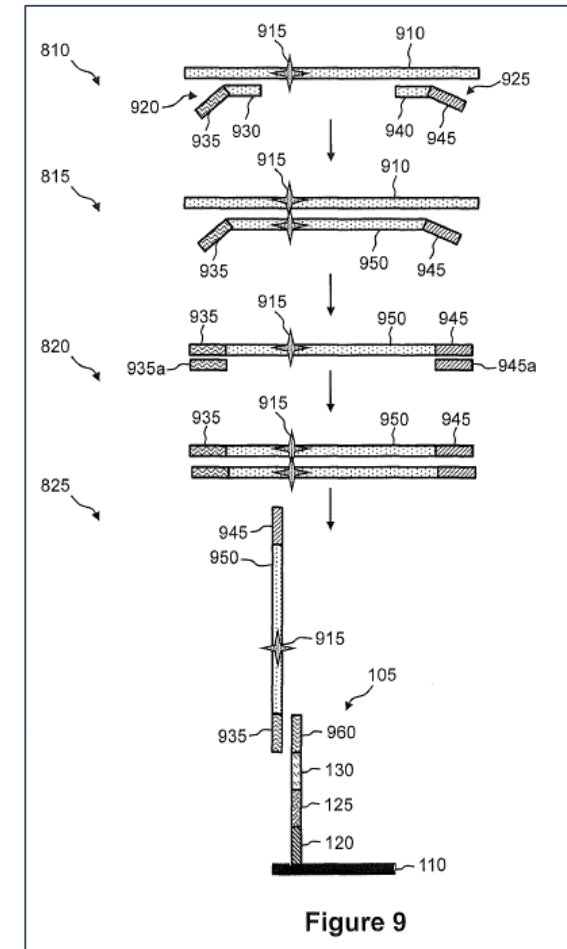
Dr. Spellman:

“So-456 teaches every element of claims 1 and 16 under NanoString’s interpretation.”

EX1002, ¶217

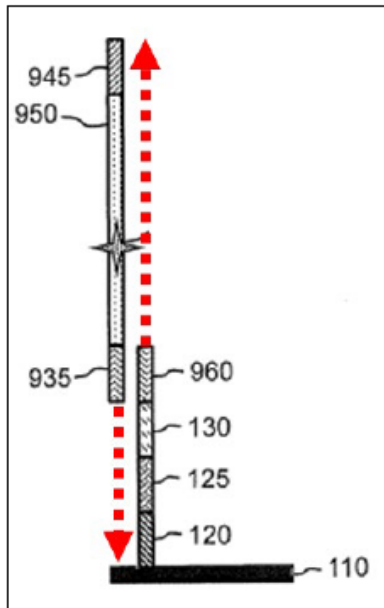
“As I explained in my first declaration and reiterate above in Section V.C, **a POSA would have collected** all of the molecules So-456 labeled as “950” in Figure 9—including the **original ligated probe** bound to the target DNA and the amplicons of the original ligated probe. EX1002, ¶¶131-137, 166-169.”

EX1055, ¶78



EX1005, Fig. 9

The claimed extension steps would have been obvious over So-456



EX1002, ¶230

- Extension enzymes, such as DNA polymerase, automatically extend from all available 3'-ends
- So-456 confirms that both *extension products* can be used for *downstream library generation*
- A POSA would have been motivated to practice the claimed extension step with a reasonable expectation of success because:
 - 1) So-456's methods create a sequencing library of molecules that are tagged with spatial barcodes,
 - 2) So-456 provides ample guidance and direction, and
 - 3) extending captured oligonucleotides and capture probes was a well-known, obvious, and routine technique for incorporating the spatial barcode

Pet., 39-45; *Pet. Reply*, 27-29; EX1002, ¶¶217-240; EX1055, ¶¶77-81

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Thank you