PATENT COOPERATION TREATY

To: BRIAN P. HOPKINS
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SUITE 700
ATTN: PATENT GROUP
WASHINGTON, DC 20004

PCT
WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43bis.1)

Date of mailing
(day/month/year) 12 FEB 2016

FOR FURTHER ACTION
See paragraph 2 below

<table>
<thead>
<tr>
<th>International application No.</th>
<th>International filing date (day/month/year)</th>
<th>Priority date (day/month/year)</th>
</tr>
</thead>
</table>

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - G01N 27/447; 30/02, 15/02; C07H 21/04 (2016.01)
CPC - B03C 5/005; G01N 27/44791; B01L 3/5027; C07H 21/04

Applicant SAGE SCIENCE, INC.

1. This opinion contains indications relating to the following items:
   - Box No. I Basis of the opinion
   - Box No. II Priority
   - Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - Box No. IV Lack of unity of invention
   - Box No. V Reasoned statement under Rule 43bis 1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - Box No. VI Certain documents cited
   - Box No. VII Certain defects in the international application
   - Box No. VIII Certain observations on the international application

2. FURTHER ACTION
   If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1 bis(b) that written opinions of this International Searching Authority will not be so considered.

   If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

   For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US
Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-8300

Date of completion of this opinion 01 February 2016

Authorized officer: Lee W. Young
PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (January 2015)
1. With regard to the language, this opinion has been established on the basis of:
   ☒ the international application in the language in which it was filed.
   ☐ a translation of the international application into ________________ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the rectification of an obvious mistake authorized by or notified to this Authority under Rule 91 (Rule 43bis.1(a)).

3. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this opinion has been established on the basis of a sequence listing:
   a. ☐ forming part of the international application as filed:
      ☐ in the form of an Annex C/ST.25 text file.
      ☐ on paper or in the form of an image file.
   b. ☐ furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
      ☐ in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).
      ☐ on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:
Box No. III  Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

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<tr>
<td>The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:</td>
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<tr>
<td>☐</td>
<td>the entire international application.</td>
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<tr>
<td>☒</td>
<td>claims Nos. 4-6</td>
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because:

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<tr>
<td>☐</td>
<td>the said international application, or the said claims Nos. _______ relate to the following subject matter which does not require an international search (specify):</td>
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<tr>
<td>☒</td>
<td>the description, claims or drawings (indicate particular elements below) or said claims Nos. 4-6 are so unclear that no meaningful opinion could be formed (specify):</td>
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Claims 4-6 are multiple dependent claims and are not drafted according to the second and third sentences of PCT Rule 6.4(a)

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<td>☐</td>
<td>the claims, or said claims Nos. _______ are so inadequately supported by the description that no meaningful opinion could be formed (specify):</td>
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<tr>
<td>☒</td>
<td>no international search report has been established for said claims Nos. 4-6</td>
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<tr>
<td>☐</td>
<td>a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:</td>
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<tr>
<td>☐</td>
<td>furnish a sequence listing in the form of an Annex C/ST.25 text file, and such listing was not available to the International Searching Authority in the form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.</td>
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<tr>
<td>☐</td>
<td>furnish a sequence listing on paper or in the form of an image file complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in the form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.</td>
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<tr>
<td>☐</td>
<td>pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13ter.1(a) or (b).</td>
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<tr>
<td>☐</td>
<td>See Supplemental Box for further details.</td>
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Form PCT/ISA/237 (Box No. III) (January 2015)
1. In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has, within the applicable time limit:
   - paid additional fees.
   - paid additional fees under protest and, where applicable, the protest fee.
   - paid additional fees under protest but the applicable protest fee was not paid.
   - not paid additional fees.

2. This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is
   - not complied with.

   This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

   Group I: Claims 1-3, 7-15, drawn to a method of manipulating DNA molecules by providing a plurality of DNA molecules having a first set of values for their radii of gyration.

   Group II: Claims 16-21, drawn to a system for manipulating DNA comprising a deterministic lateral displacement array device.

   The inventions listed as Groups I and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

   Special Technical Features:
   - Group I has the special technical feature of a method step of providing a solution of a plurality of DNA molecules having a set of first values for their radii of gyration, said first radii values varying in proportion to the molecular weight of each DNA molecule, under a zero transport velocity, not required by Group II.
   - Group II has the special technical feature of a deterministic lateral displacement (DLD) array device and system, wherein the dimensions of the array are configured to fractionate DNA molecules on the basis of size, such that DNA molecules greater than a critical size move in a first direction through the array, and DNA molecules less than a critical size move in a second direction through the array.

   Common Technical Feature:
   - Groups I and II have the common technical feature of maintaining DNA molecules in a spherical shape under a transport velocity.

   However, said common technical feature does not represent a contribution over the prior art, and is anticipated by the publication titled "Compression and self-entanglement of single DNA molecules under uniform electric field" by TANG et al. [hereinafter "Tang"] [published 27 September 2011 in Proc Nat Acad Sci Vol 108 No 39 Pages 16153-1158].

   Tang teaches maintaining DNA molecules in a spherical shape under a transport velocity (pg 1653 col 1 para 2; "Electric fields are a convenient mode to transport DNA as they scale favorably with device dimensions"); pg 1653 col 2 para 3; "Isotropic Compression of DNA Under Uniform Electric Field. We experimentally examine the conformation of dilute fluorescently labeled DNA molecules under uniform electric fields in 2 um tall by 200 um wide straight channels. Fig. 1A shows fluorescent images of individual T4 DNA molecules (165.6 kbp) at equilibrium (i.e., no electric field) and under a uniform DC electric field. Surprisingly, the electric field induces a strong isotropic compression of the DNA: while the equilibrium coil remains relatively extended and highly anisotropic, the T4 DNA becomes significantly more compact under an electric field of 94 V/cm, and is further compressed into a spherical globule by a stronger electric field of 250 V/cm "]

   As the common technical feature was known in the art at the time of the invention, this cannot be considered a common special technical feature that would otherwise unify the groups. The inventions lack unity with one another.

   Therefore, Groups I and II lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.

   Note concerning Item 4: Claims 4-6 are multiple dependent claims and are not drafted according to the second and third sentences of PCT Rule 6.4(a).

4. Consequently, this opinion has been established in respect of the following parts of the international application:
   - all parts.
   - the parts relating to claims Nos. 1-3.
WITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 15/56104

Box No. V  Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

<table>
<thead>
<tr>
<th>1. Statement</th>
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<tbody>
<tr>
<td><strong>Novelty (N)</strong></td>
<td>Claims 3, 7-11, 15-21, 1-2, 12-14</td>
</tr>
<tr>
<td><strong>Inventive step (IS)</strong></td>
<td>Claims NONE</td>
</tr>
<tr>
<td><strong>Industrial applicability (IA)</strong></td>
<td>Claims 1-3, 7-21, NONE</td>
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2. Citations and explanations:
Claim 1, 2, 12-14 lack novelty under PCT Article 33(2) as being anticipated by “Continuous particle separation through deterministic lateral displacement” by HUANG et al. (hereinafter “Huang”).

As to claim 1, Huang teaches a method for manipulating DNA molecules for use in a microfluidic device, the method comprising: providing a solution of a plurality of DNA molecules having a set of first values for their radii of giration, said first radii values varying in proportion to the molecular weight of each DNA molecule, under a zero transport velocity (Abstract; We report on a microfluidic particle-separation device that makes use of the asymmetric bifurcation of laminar flow around obstacles. A particle chooses its path deterministically on the basis of its size. All particles of a given size follow equivalent migration paths, leading to high resolution. The microspheres of 0.8, 0.9, and 1.0 micrometers that were used to characterize the device were sorted in 40 seconds with a resolution of approximately nanometers; pg 989 col 3 para 1 continued to pg 990 col 1 para 1; “we prepared bacterial artificial chromosomes, a widely used DNA source for many genomics projects, from Escherichia coli by means of standard protocols. These molecules tend to coi up and can thus be APPROXIMATED AS SOFT SPHERES. Electric fields were used to drive the molecules through the matrix. These fields created electric currents, which were bifurcated in the matrix in the same manner as fluid flow in the earlier example with beads. Bacterial artificial chromosomes of 61 and 158 kb were separated into two bands, with widths that corresponded to CVs of approximately 12 and 5% (molecular weight), respectively; pg 989 col 3 Fig 4”) substantially maintaining the DNA molecules in a spherical shape under a transport velocity (pg 990 col 1 para 1; “These fields created electric currents, which were bifurcated in the matrix in the same manner as fluid flow in the earlier example with beads. Bacterial artificial chromosomes of 61 and 158 kb were separated into two bands, with widths that corresponded to CVs of approximately 12 and 5% (molecular weight), respectively (Fig. 4) (26), in a field of approximately 12 V/cm, creating a migration speed of approximately 20 um/s. Higher fields result in reduced separation, possibly because of random deformation and stretching of DNA, which are caused by collisions with obstacles”).

As to claim 2, Huang teaches a method for manipulating DNA molecules in a microfluidic device, the method comprising: providing a solution of a plurality of DNA molecules having a set of first values for their radii of giration (pg 989 col 3 para 1 continued to pg 990 col 1 para 1), said first radii values varying in proportion to the molecular weight of each DNA molecule, under a zero transport velocity (pg 989 col 3 para 1 continued to pg 990 col 1 para 1; abstract), and providing conditions wherein the DNA molecules: substantially maintain their first radii values under a transport velocity, and/or decrease their radii of giration to a set of second values for their radii of giration, said second radii of giration varying in proportion to the molecular weight of each DNA molecule under a transport velocity (pg 989 col 3 para 1 continued to pg 990 col 1 para 1; pg 989 fig 4).

As to claim 12, Huang further teaches flowing the solution through a microfluidic device. (pg 989 col 3 para 1 continued to pg 990 col 1 para 1)

As to claim 13, Huang further teaches that the microfluidic device is a deterministic lateral displacement (DLD) array (abstract and titled; deterministic lateral displacement).

As to claim 14, Huang further teaches that the microfluidic device manipulates the DNA molecules, wherein manipulation comprises fractionation by size (pg 989 fig 4).

Claim 15 lacks an inventive step under PCT Article 33(3) as being obvious over Huang.

As to claim 15, concerning the chemical modification comprises enzymatic modification, the claim is obvious based on the teaching of Huang using bacterial artificial chromosomes (pg 989 col 3 para 1), which were well-known in the art to be artificial constructs which required the use of restriction enzymes and ligase to make them.

-----continued in Supplemental Box-----
Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:
Box V-2 (Citations and Explanations)

Claim 3, 7, 16-21 lack an inventive step under PCT Article 33(3) as being obvious over Huang, in view of the publication titled "Interaction between DNA and Trimethyl-Ammonium Bromides with Different Alkyl Chain Lengths" by CHENG et al. (hereinafter "Cheng").

As to claim 3, Huang teaches use of a common biological buffer 1/2x Tris-borate-EDTA (i.e., 1/2 x TBE) (pg 989 col 3 para 1 footnote 27: We used 1/2x TBE as the electrophoresis buffer). Huang does not teach maintaining the spherical shape of the DNA molecules, or maintaining and/or decreasing the set of first values for their radi of gyration of the DNA molecules comprises adding an amount of DNA condensation agent to the solution. However, Cheng teaches that addition of Cetyltrimethylammonium (CTAB) as a DNA condensing agent converted DNA molecules from random coil to a spherical shape (pg 7 col 1 para 3; The competition between the driving terms and the resisting ones determines the final conformation of DNA-surfactant complexes. If hydrophobic force overcomes the resisting terms, spherical compacted particles are the favorable conformations, which are the cases for the surfactants with sufficient long chain e.g. CTAB; pg 2 col 2 para 3 continued to pg 4 col 1 para 1; The DNA samples with varying concentrations of surfactants added and incubated were then measured by DLS to obtain their size distribution curves. Typical intensity weighted distribution curves in the presence of different concentrations of surfactants are shown in Figure 2. For a CTAB concentration of 5 μM, two peaks with a hydrodynamics radius of 42.8nm and the other 223.4nm appeared; this observation suggests the compacted DNA molecules coexist with DNA coils, as revealed by previous studies. Increasing the CTAB concentration leads to the disappearance of the larger-radius peak; see p3 fig 2A.). An artisan of ordinary skill in the art would have recognized that condensing agents, such as CTAB, favored a spherical shape rather than an unruly random coil shape for the DNA [would lead to clogging], as taught by Cheng, and thus would have been useful in size separation of spheres, as used in the study of Huang. Consequently it would have been obvious for one of ordinary skill in the art to combine manipulating DNA molecules in a microfluidic device, as taught by Huang, with adding a DNA condensing agent to maintain the spherical shape of the DNA, because spherical shaped objects were more well-behaved in size separation devisors.

As to claim 7, Cheng teaches that the DNA condensation agent is Cetyltrimethylammonium bromide (CTAB) (pg 2 col 2 para 3 continued to pg 4 col 1 para 1; abstract).

As to claim 16, Huang teaches system for manipulating DNA in a microfluidic environment, comprising: a deterministic lateral displacement (DLD) array (abstract, title: deterministic lateral displacement), wherein the dimensions of the array are configured to fractionate DNA molecules on the basis of size (pg 989 fig 4A,B), such that DNA molecules greater than a critical size move in a first direction through the array, and DNA molecules less than a critical size move in a second direction through the array (pg 989 col 3 para 1 continued to pg 990 col 1 para 1; pg 986 fig 4A, C), the transport velocity is configured such that the DNA molecules retain an approximately spherical conformation (pg 989 col 3 para 1; soft spheres) as they pass through the microfluidic array (pg 989 col 3 para 1 continued to pg 990 col 1 para 1). Huang does not teach a DNA condensation reagent or configuring the condensation reagent so that the DNA molecules retain an approximately spherical conformation. However, Cheng teaches a DNA condensation agent (abstract; CTAB) and configuring the condensation reagent so that the DNA molecules retain an approximately spherical conformation (pg 7 col 1 para 3; pg 2 col 2 para 3 continued to pg 4 col 1 para 1; see p3 fig 2A). An artisan of ordinary skill in the art would have recognized that condensing agents, such as CTAB, favored a spherical shape rather than an unruly random coil shape for the DNA [would lead to clogging], as taught by Cheng, and thus would have been useful in size separation of spheres, as used in the study of Huang. Consequently, It would have been obvious for one of ordinary skill in the art to combine all claim elements, with the exception of a DNA condensation reagent, as taught by Huang, with a DNA condensation reagent, as taught by Cheng, configuring the condensation reagent so that the DNA molecules retain an approximately spherical conformation, as taught by Cheng, in view of the perspective of an artisan of ordinary skill, because it would have enabled a system for manipulating DNA in a microfluidic environment.

As to claim 17, Huang further teaches that the transporter comprises a flow (pg 989 col 3 para 1; "These fields created electric currents, which were bifurcated in the matrix in the same manner as fluid flow in the earlier example with bocdo").

As to claim 18, Huang further teaches that the transporter comprises at least one electric field (pg 989 fig 4A; Bacterial artificial chromosome DNAs (61 and 158 kb at 2 and 1 μm/g, respectively) were electrophoresed through the device with a field of 12 V/cm")

As to claim 19, Huang further teaches that the transporter comprises a combination of a flow and at least one electric field (pg 989 fig 4).

As to claim 20, concerning the DLD array is configured with a critical size for fractionation, expressed as a particle diameter of between 0.1 and 3 μm, Huang teaches particles of 0.8-1.0 μm (abstract; "The microspheres of 0.8, 0.9, and 1.0 micrometers that were used").

As to claim 21, Cheng further teaches that the condensation agent causes the DNA molecules of the sample to adopt a more compact size (Cheng pg 3 fig 2A) when passing through the DLD array at the transport velocity (Huang; pg 989 col 3 para 1 and pg 989 fig 4).

-----continued in next Supplemental Box-----
Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:
previous Supplemental Box

Claims 8-11 lack an inventive step under PCT Article 33(3) as being obvious over Huang, in view of Cheng, in final view of US 5,707,812 A to HORN et al. (hereinafter "Horn").

As to claims 8-11, Cheng teaches using a CTAB at between 10 uM-1000 uM (pg 3 fig 3A); where an artisan of ordinary skill would have known that CTAB had a known molecular weight of 364 daltons, such that 1000 uM was equivalent to 0.308 % wt/vol. Neither Cheng nor Huang teaches the amount of DNA condensation agent is between about 1 % to about 40% wt/vol, 1 % to about 20% wt/vol, 5 % to about 20% wt/vol, 10% to about 20% wt/vol. However, Horn teaches the amount of DNA condensation agent is between about 1 % to about 40% wt/vol, 1 % to about 20% wt/vol, 5 % to about 20% wt/vol, 10% to about 20% wt/vol. (col 4 In 37-40; "The DNA pellet is resuspended in column buffer prior to column chromatography. This buffer contains ref, either alone or in combination. Typically, the concentration of PEG in the DNA solution is from or about 0.1% and to or about 4% (w/v), preferably about 1% (w/v)/"). Optimization of parameters is a routine practice that would be obvious for a person of ordinary skill in the art to employ. It would have been customary for an artisan of ordinary skill to determine the optimal amount of each ingredient needed to achieve the desired results. Consequently, it would have been obvious for one of ordinary skill in the art to combine using a DNA condensation agent, as taught by Cheng, with the DNA condensation agent is PEG at between about 1 % to about 40% wt/vol, 1 % to about 20% wt/vol, 5 % to about 20% wt/vol, 10% to about 20% wt/vol, as taught by Horn, because it would have enabled maintaining the DNA particles in spherical form in the obstacle microfluidic device.

Claims 1-3, 7-21 have industrial applicability as defined in PCT Article 33(4) because the subject matter can be made or used by industry.