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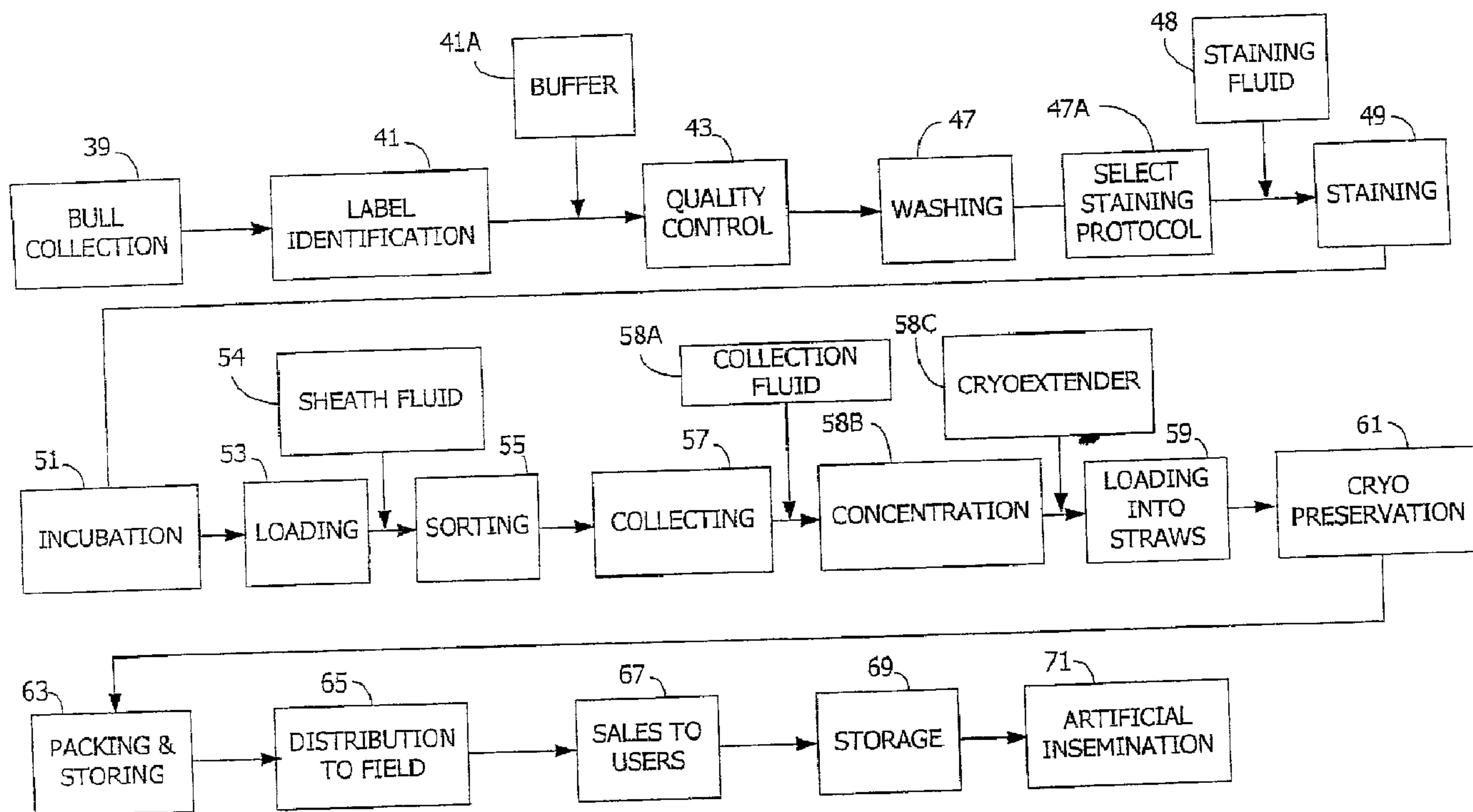
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C12M 1/34 (2006.01), *G01N 15/10* (2006.01),
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(71) Demandeur/Applicant:
 INGURAN, LLC, US

(72) Inventeurs/Inventors:
 DURACK, GARY, US;
 HATCHER, JEREMY T., US;
 WESTFALL, LON A., US;
 HELBLING, DAVID R., US;
 WALLACE, JEFFREY D., US;
 VANDRE, GARY P., US;

(74) Agent: MCFADDEN, FINCHAM

(54) Titre : DISPOSITIFS, PROCÉDES ET PROCESSUS PERMETTANT DE TRIER DES PARTICULES ET D'OBTENIR
 DU SPERME ANIMAL PRODUISANT UNE PROGENITURE DE SEXE DONNE
 (54) Title: APPARATUS, METHODS AND PROCESSES FOR SORTING PARTICLES AND FOR PROVIDING SEX-
 SORTED ANIMAL SPERM



(57) Abrégé/Abstract:

Apparatus for sorting stained particles in a fluid stream according to one or more characteristics of the particles, said system comprising: a. a fluid delivery system for delivering a fluid stream containing the particles to a first location; b. an electromagnetic radiation source for delivering a beam of electromagnetic radiation to the first location for exciting particles in the fluid stream to produce fluorescence emissions; c. an epi-illumination optics system including a focusing lens, the optics system being operable to direct the beam of electromagnetic radiation through the focusing lens in a forward direction along a beam axis intersecting particles in the fluid stream at said first location so that said particles pass through the beam, resulting in the fluorescence

(72) **Inventeurs(suite)/Inventors(continued)**: DIDION, BRADLEY, US; NAYAK, NIRAJ V., US; ANZAR, MUHAMMAD, US; LUDWIG, CINDY L., US; GRAHAM, JEFFREY A., US; CROWLEY, KATHLEEN S., US

(57) **Abrégé(suite)/Abstract(continued)**:

emissions from the particles being directed along said beam axis in a rearward direction; d. a photodetector for the detecting fluorescence emissions from the excited particles; and e. a processor in communication with the photodetector to classify the particles according to the one or more characteristics based upon the fluorescence emissions of the particles and to sort the particles according to the classification of particles.

ABSTRACT

Apparatus for sorting stained particles in a fluid stream according to one or more characteristics of the particles, said system comprising:

- a. a fluid delivery system for delivering a fluid stream containing the particles to a first location;
- b. an electromagnetic radiation source for delivering a beam of electromagnetic radiation to the first location for exciting particles in the fluid stream to produce fluorescence emissions;
- c. an epi-illumination optics system including a focusing lens, the optics system being operable to direct the beam of electromagnetic radiation through the focusing lens in a forward direction along a beam axis intersecting particles in the fluid stream at said first location so that said particles pass through the beam, resulting in the fluorescence emissions from the particles being directed along said beam axis in a rearward direction;
- d. a photodetector for the detecting fluorescence emissions from the excited particles; and
- e. a processor in communication with the photodetector to classify the particles according to the one or more characteristics based upon the fluorescence emissions of the particles and to sort the particles according to the classification of particles.

CLAIMS:

1. Apparatus for sorting stained particles in a fluid stream according to one or more characteristics of the particles, said system comprising:
 - a. a fluid delivery system for delivering a fluid stream containing the particles to a first location;
 - b. an electromagnetic radiation source for delivering a beam of electromagnetic radiation to the first location for exciting particles in the fluid stream to produce fluorescence emissions;
 - c. an epi-illumination optics system including a focusing lens, the optics system being operable to direct the beam of electromagnetic radiation through the focusing lens in a forward direction along a beam axis intersecting particles in the fluid stream at said first location so that said particles pass through the beam, resulting in the fluorescence emissions from the particles being directed along said beam axis in a rearward direction;
 - d. a photodetector for the detecting fluorescence emissions from the excited particles; and
 - e. a processor in communication with the photodetector to classify the particles according to the one or more characteristics based upon the fluorescence emissions of the particles and to sort the particles according to the classification of particles.
2. The apparatus of claim 1, wherein said particles are sperm cells.
3. The apparatus of claim 2, wherein the one of the characteristics comprises the presence of an X chromosome or the presence of a Y chromosome.
4. The apparatus of claim 3, wherein the one of the characteristics comprises the presence of a live cell or a dead cell.

5. The apparatus of claim 4 further comprising an ablating laser for ablating sperm cells based upon their classification.
6. The apparatus of claim 5 further comprising a control in communication with the processor and with the ablating laser to coordinate the ablation of cells classified as sperm other than live X chromosome bearing sperm (~X).
7. The apparatus of claim 5 further comprising a control in communication with the processor and with the ablating laser to coordinate the ablation of cells classified as sperm other than live Y chromosome bearing sperm (~Y).
8. The apparatus of claim 1, wherein the stained particles comprise sperm cells stained with a DNA selective dye.
9. The apparatus of claim 1, wherein the photodetector is positioned to the rear of said focusing lens for detecting said fluorescence emissions.
10. The apparatus of claim 1, wherein said first location is inside a capillary tube.
11. A method of sorting particles contained in a fluid stream according to one or more characteristics of the particles, said method comprising:
 - a. delivering a fluid stream containing said particles to a first location;
 - b. exciting particles in the fluid stream at the first location with a beam of electromagnetic radiation to produce a fluorescence emission, wherein the beam of electromagnetic radiation has a beam axis and travels in the forward direction along the beam axis and the fluorescence emission is directed in the rearward direction;
 - c. detecting the fluorescence emissions with a photodetector;

- d. classifying the particles according to the one or more characteristics based upon the detected fluorescence emission; and
 - e. sorting particles based upon their one or more characteristics.
12. The method of claim 11, wherein said particles are sperm cells.
13. The method of claim 12, wherein the step of sorting further comprising the steps of :
- a. selecting sperm cells based upon their classification; and
 - b. ablating the selected sperm cells.
14. The method of claim 11, wherein the selected particles comprises particles classified as sperm other than live X chromosome bearing sperm ($\sim X$).
15. The method of claim 11, wherein the selected particles comprises particles classified as sperm other than live Y chromosome bearing sperm ($\sim Y$).
16. Apparatus for sorting stained particles in a fluid stream according to one or more characteristics of the particles, said system comprising:
- a. a fluid delivery system for delivering a fluid stream containing the particles to a first location;
 - b. an electromagnetic radiation source for delivering a beam of electromagnetic radiation to excite the particles at the first location;
 - c. a photodetector for detecting fluorescence emissions from the excited particles;
 - d. a processor in communication with the photodetector to classify the particles according to the one or more characteristics based

- upon the fluorescence emissions of the particles and to sort the particles according to the classification of particles;
- e. an epi-illumination optics system including a focusing lens, the optics system being operable to direct the beam of electromagnetic radiation through the focusing lens in a forward direction along a beam axis intersecting particles in the fluid stream at said first location so that said particles pass through the beam resulting in the fluorescence emissions from the cells being directed along said beam axis in a rearward direction; and
 - f. a laser in communication with the processor for ablating particles in said stream to effect sorting the particles.
17. The apparatus of claim 16, wherein said particles are cells or sperm cells.
18. The apparatus of claim 17, wherein said flow cytometry apparatus further comprises a nozzle having an interior surface configured to exert a force on the particles tending to bring them into a desired orientation prior to passing through said beam.
19. A method of sorting particles contained in a fluid stream according to one or more characteristics of the particles, said method comprising:
- a. delivering a fluid stream containing said particles to a first location;
 - b. exciting particles in the fluid stream at the first location with a beam of electromagnetic radiation to produce a fluorescence emission, wherein the beam of electromagnetic radiation has a beam axis and travels in the forward direction along the beam axis and the fluorescence emission is directed in the rearward direction;
 - c. detecting the fluorescence emissions with a photodetector;
 - d. classifying the particles according to the one or more characteristics based upon the detected fluorescence emission;

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- e. selecting particles based upon their classification; and
 - f. ablating the selected particles.
20. The method of claim 11, wherein the selected particles comprises particles classified as sperm other than live X chromosome bearing sperm ($\sim X$).
21. The method of claim 11, wherein the selected particles comprises particles classified as sperm other than live Y chromosome bearing sperm ($\sim Y$).

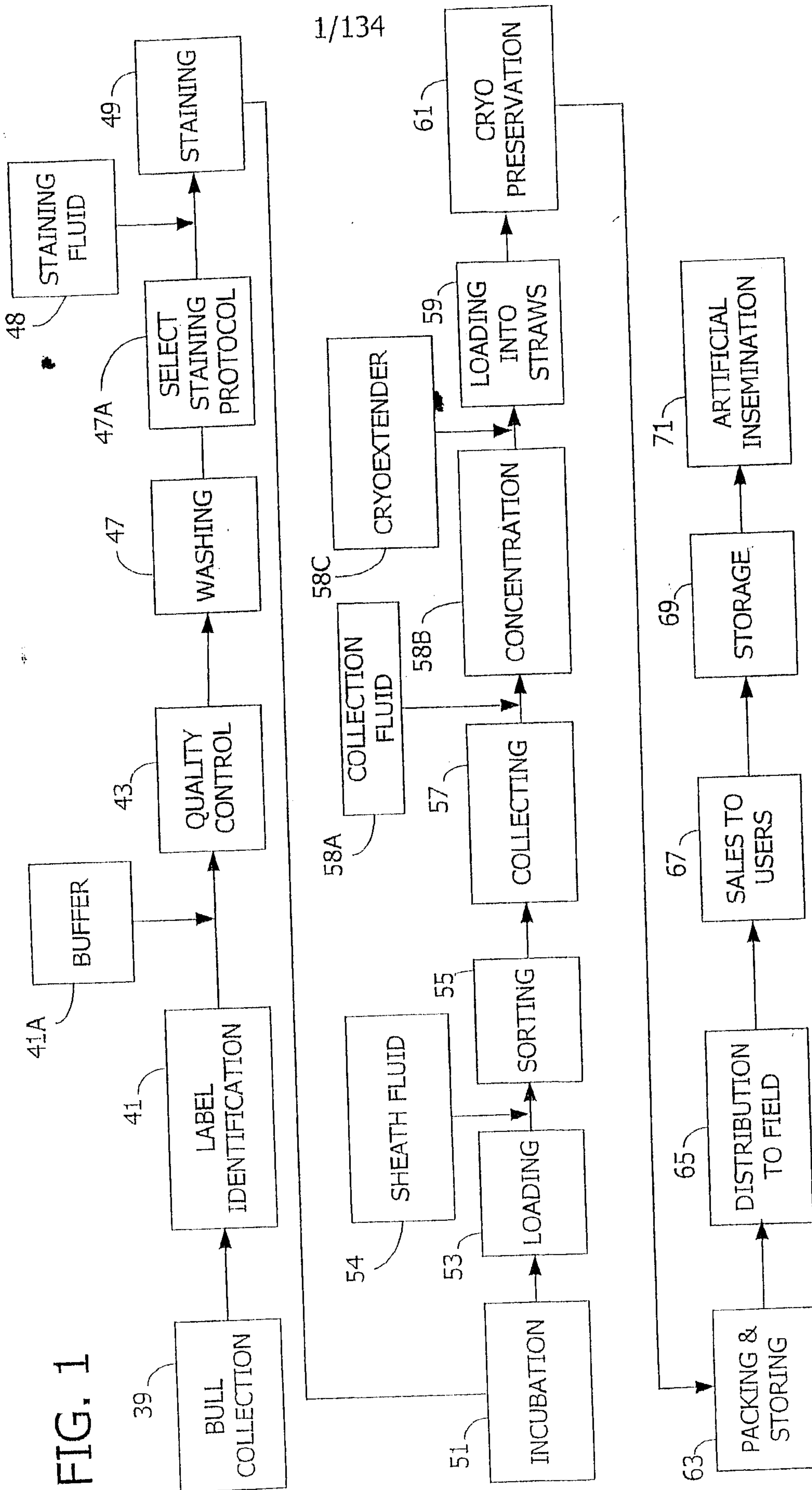
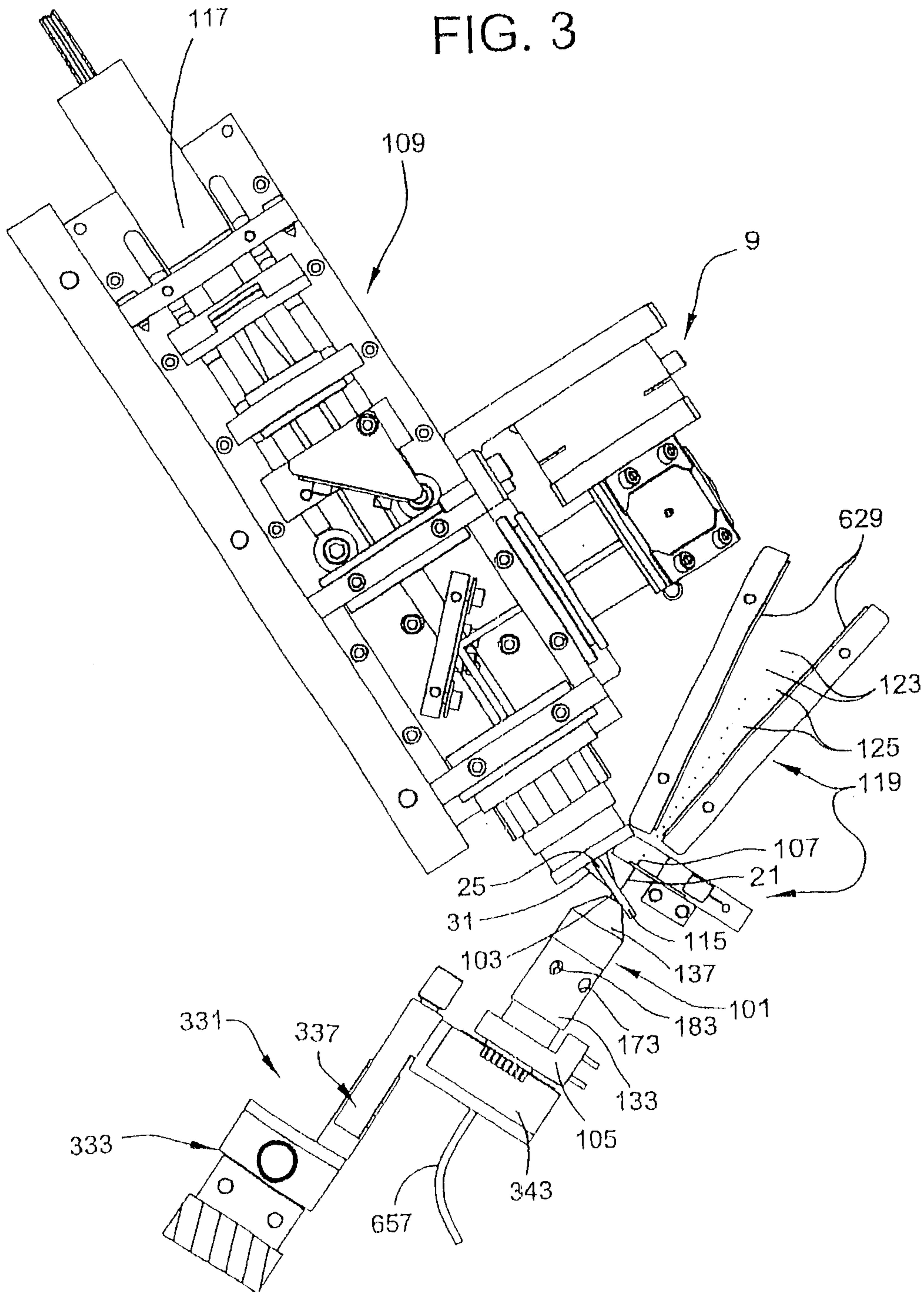


FIG. 1

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FIG. 3



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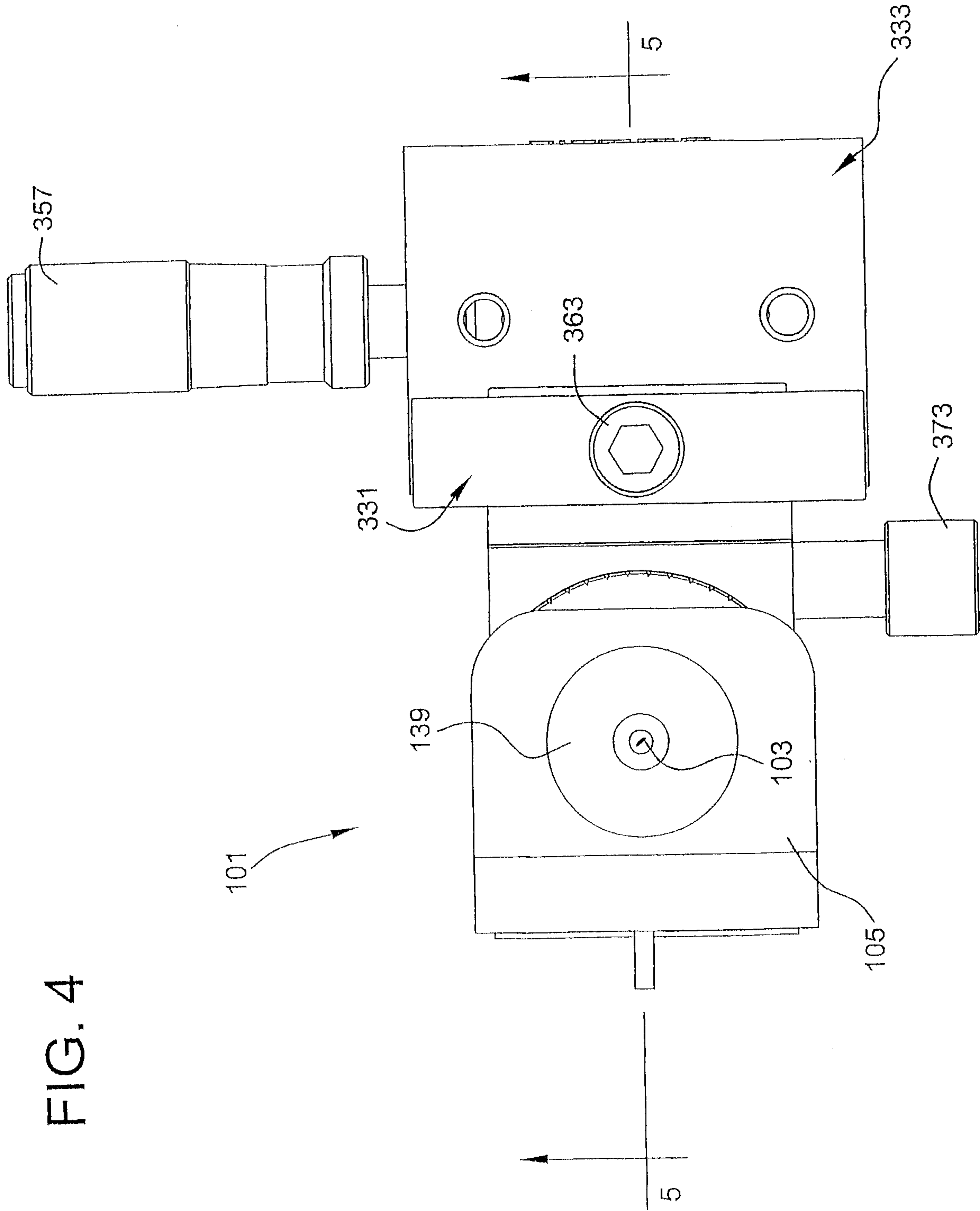
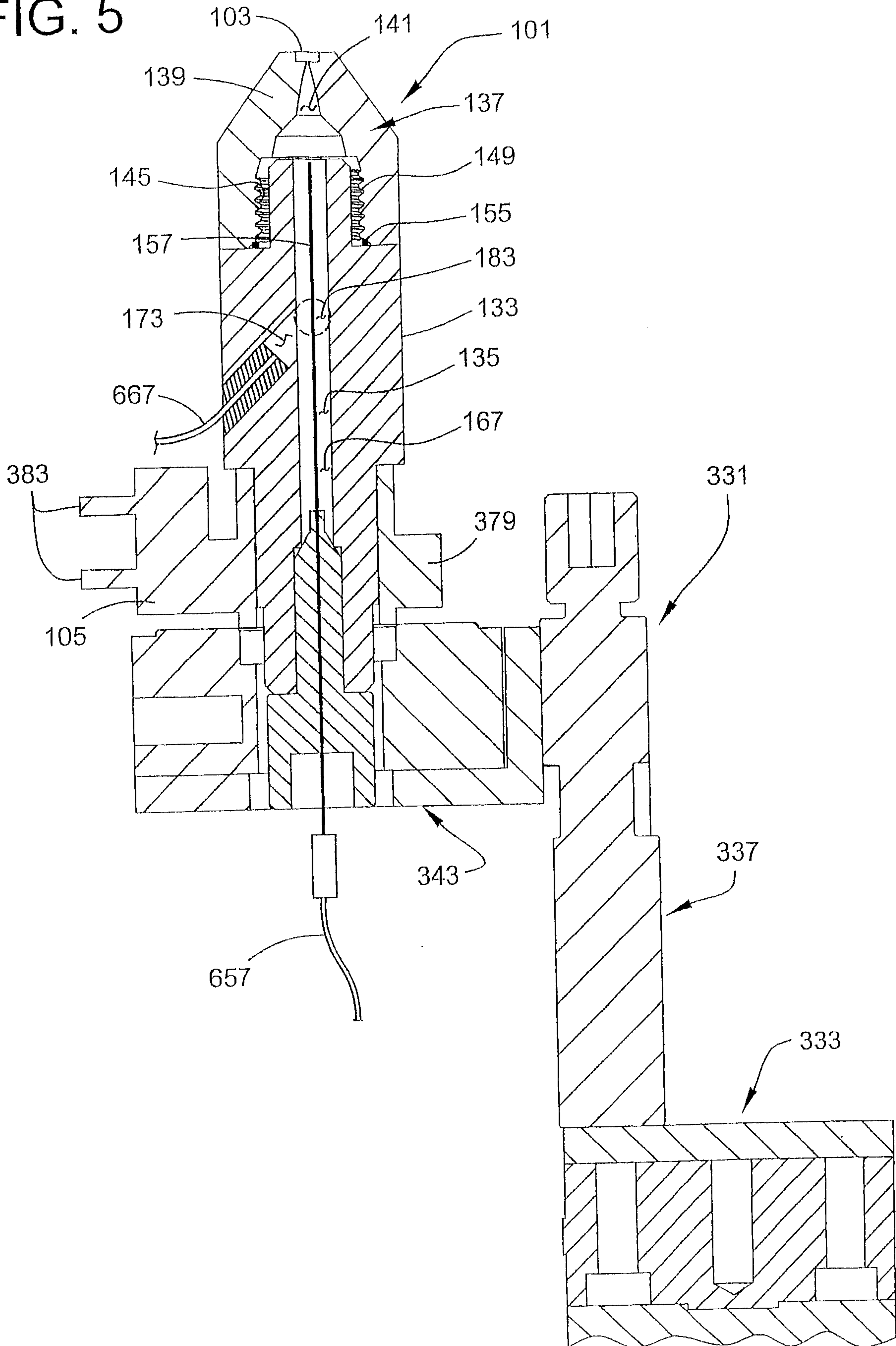


FIG. 4

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FIG. 5



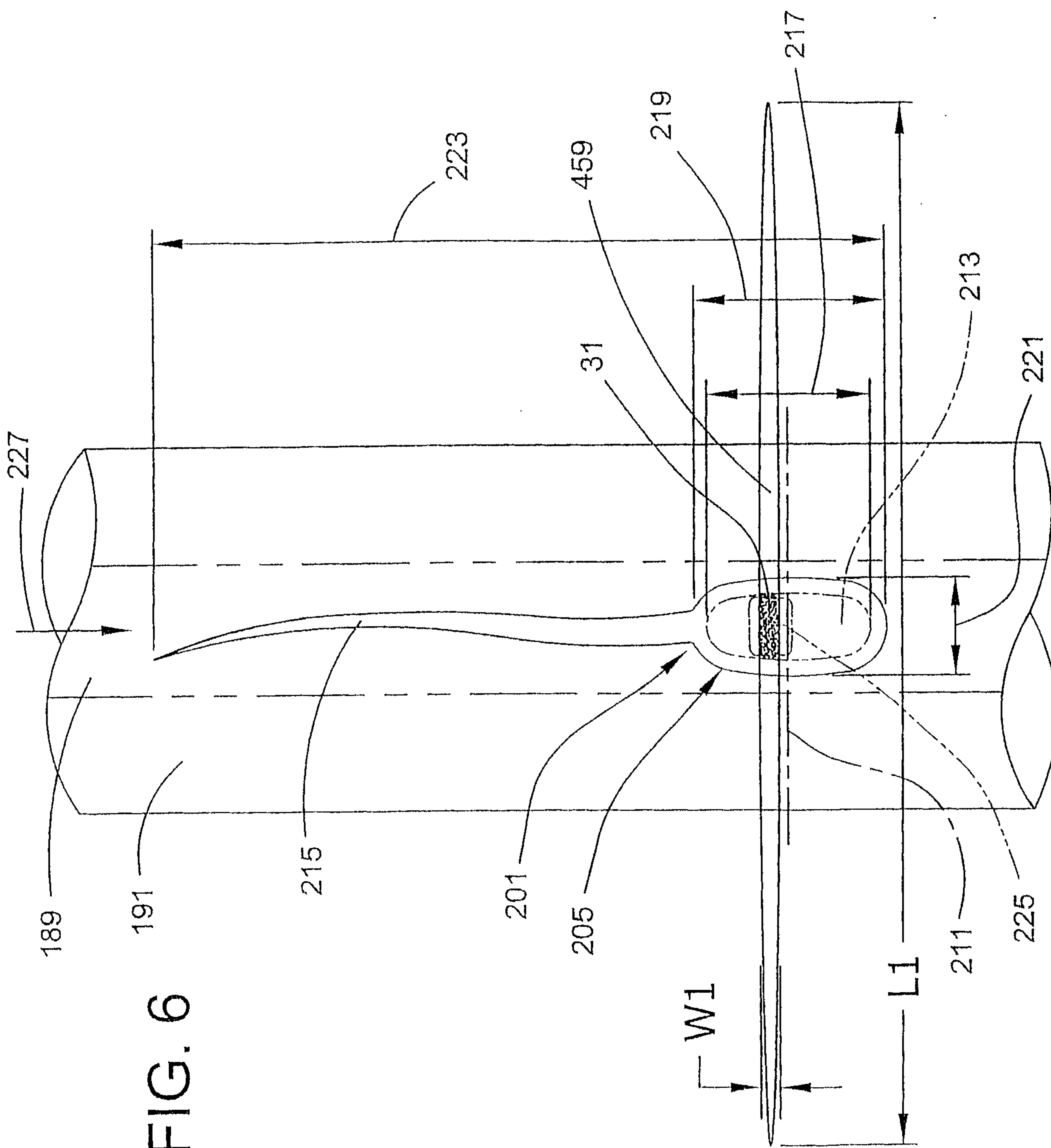


FIG. 6

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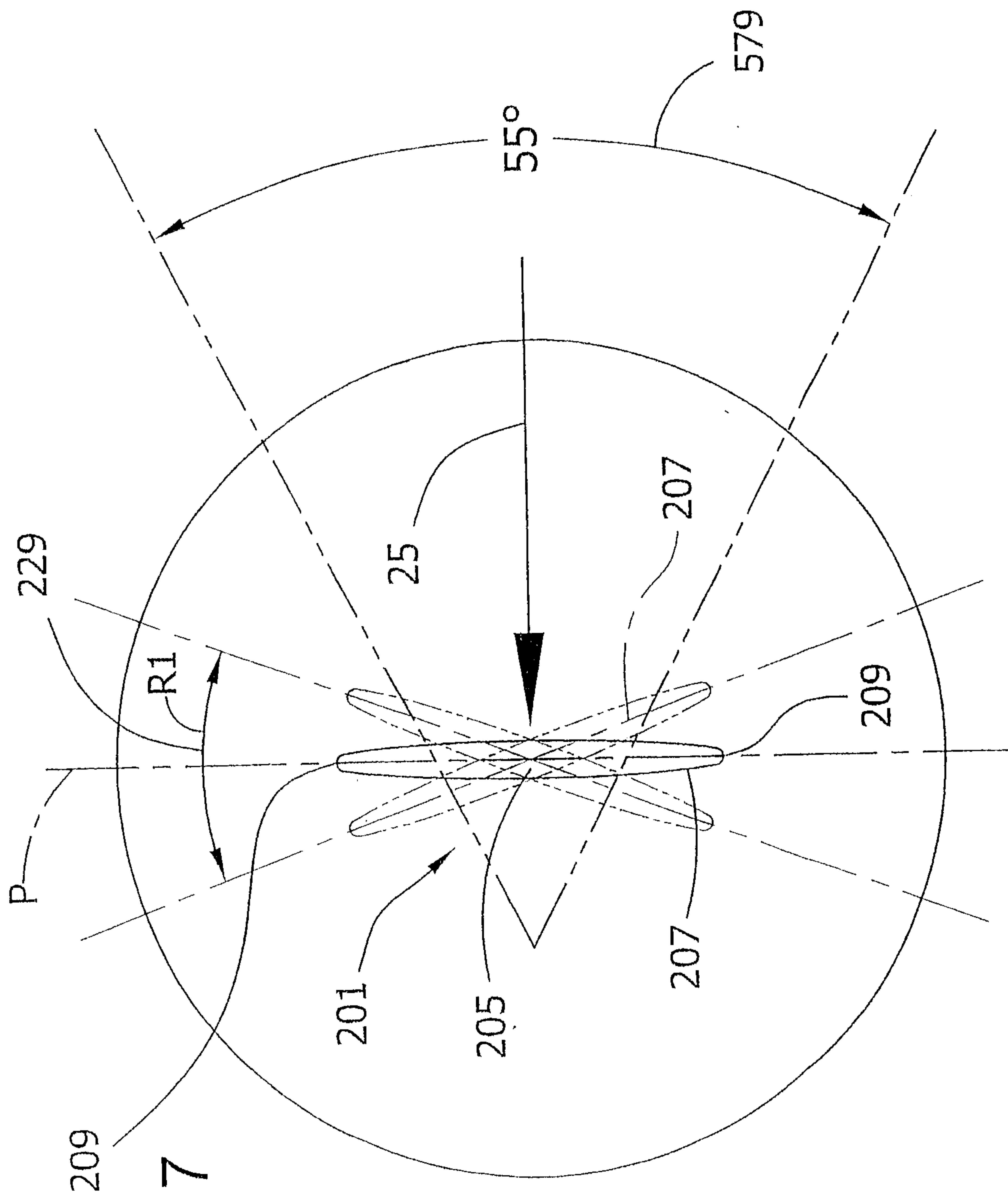


FIG. 7

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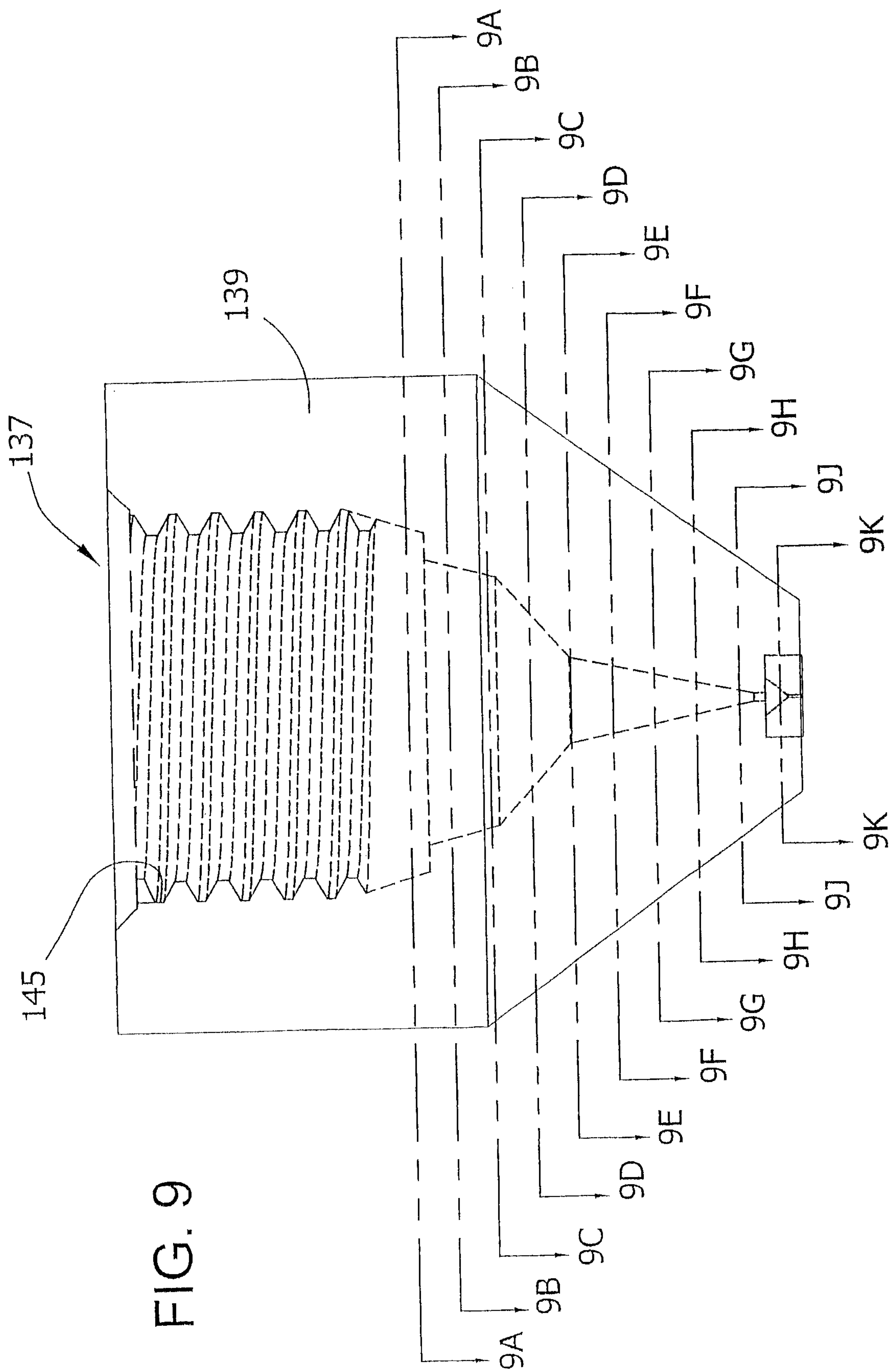


FIG. 9

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FIG. 9B

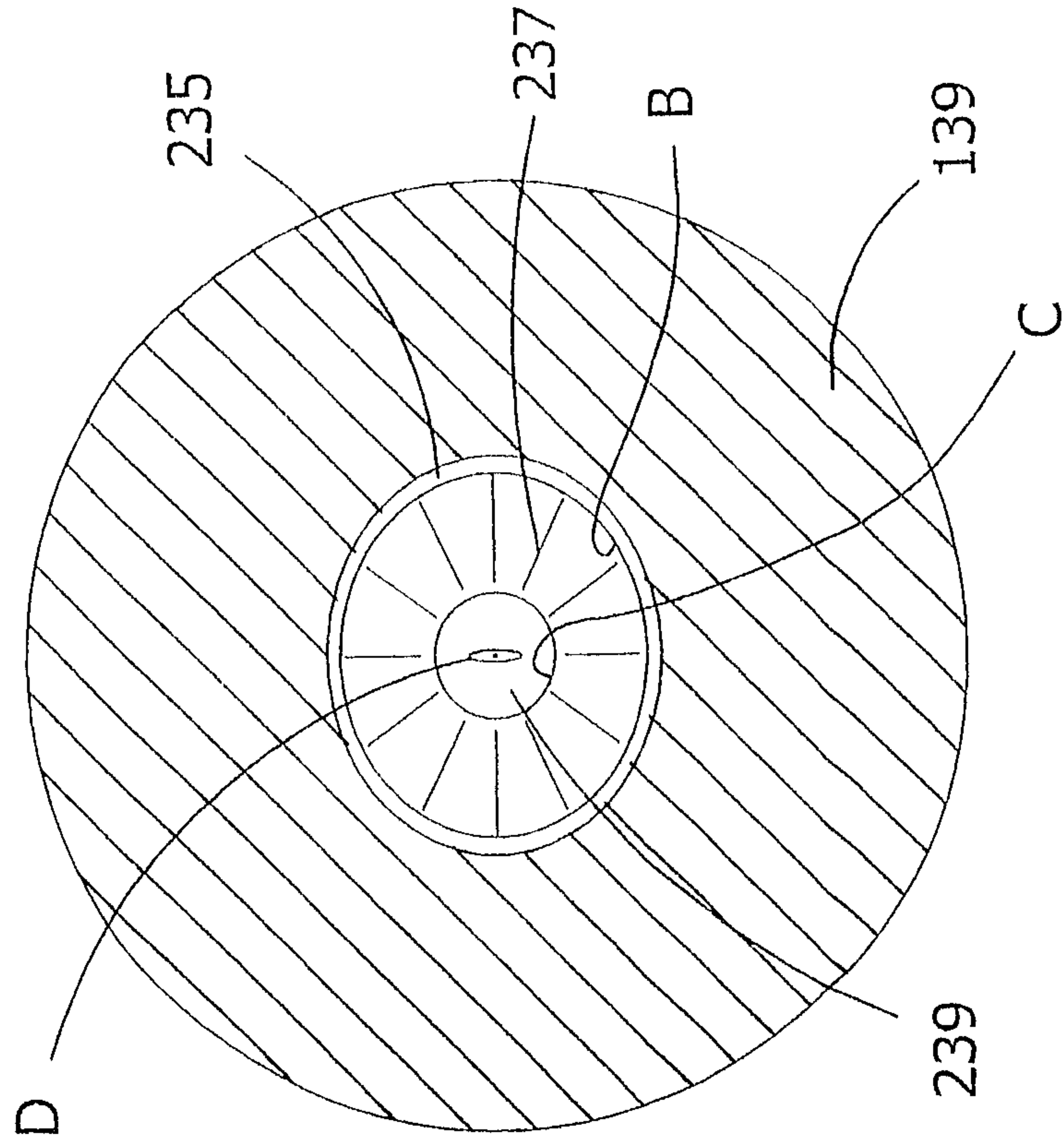
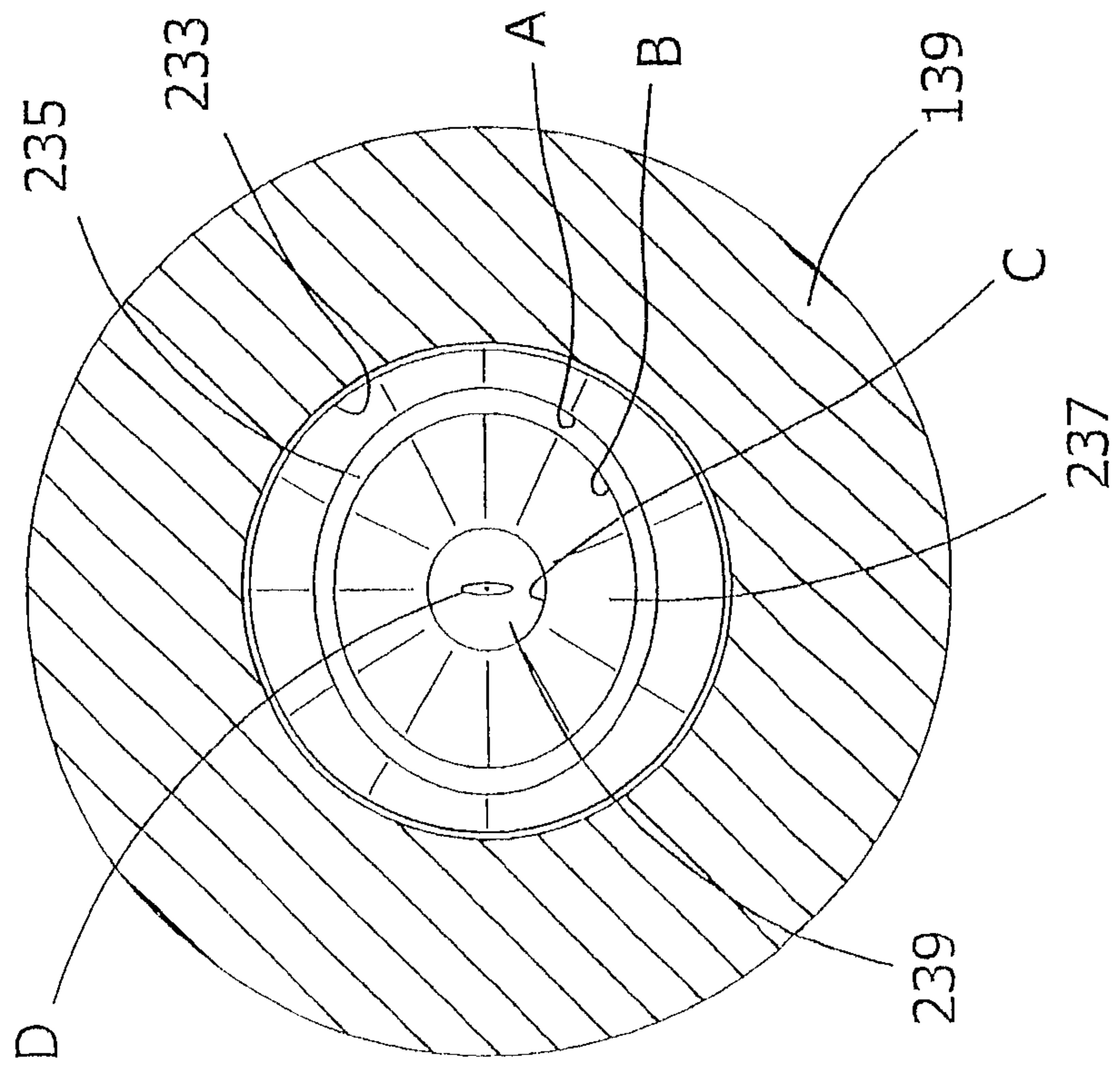


FIG. 9A



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FIG. 9D

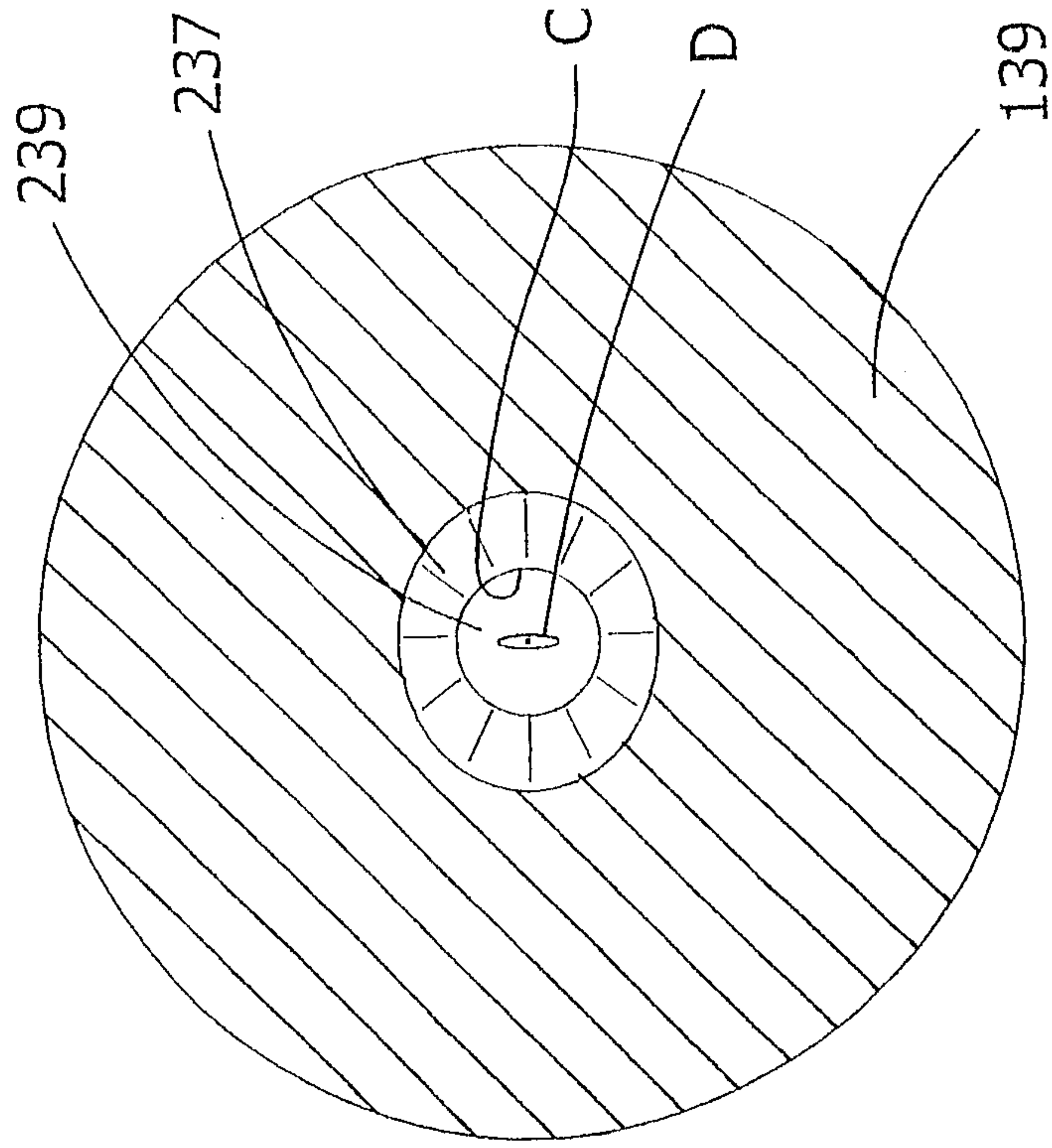
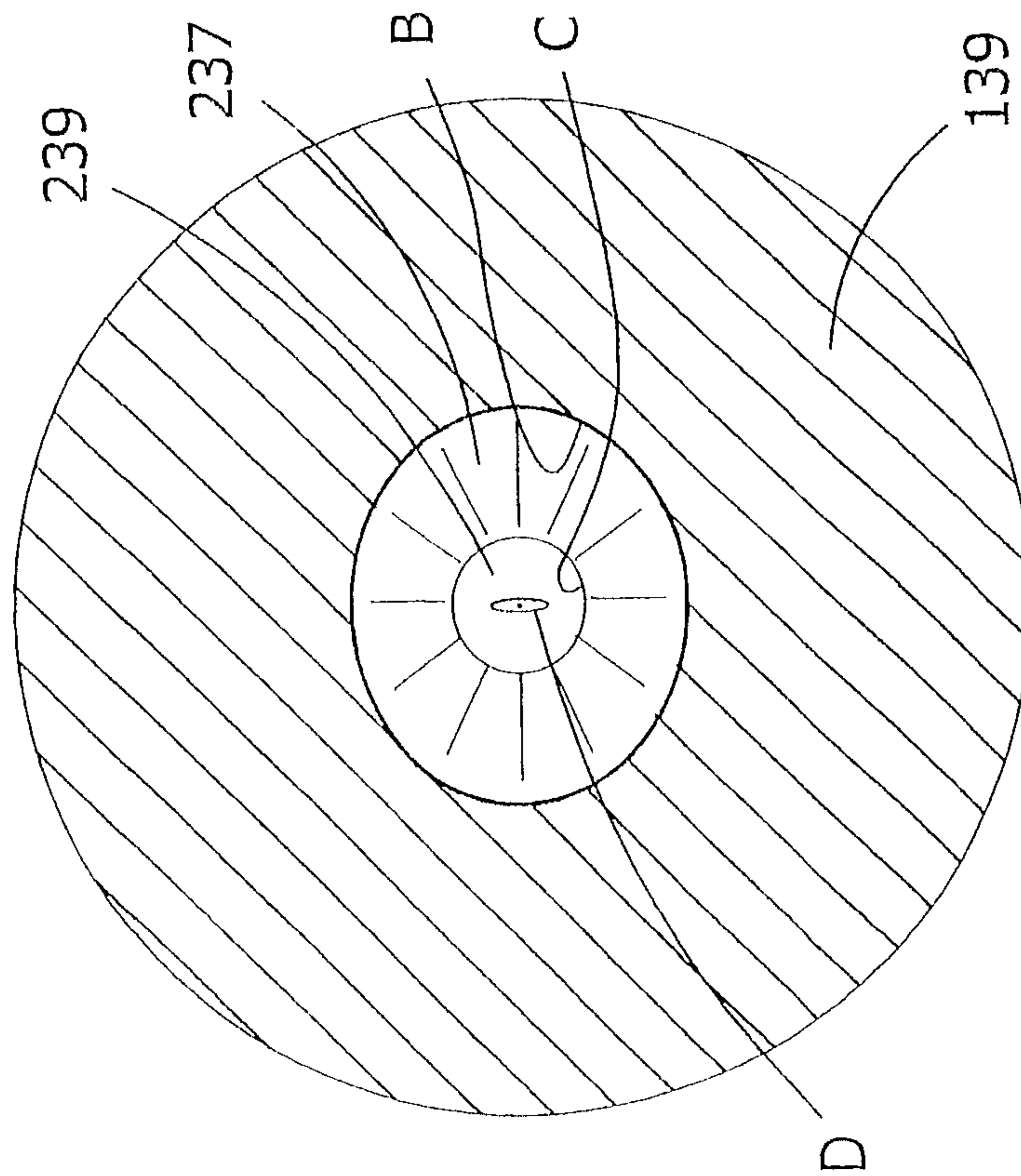


FIG. 9C



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FIG. 9F

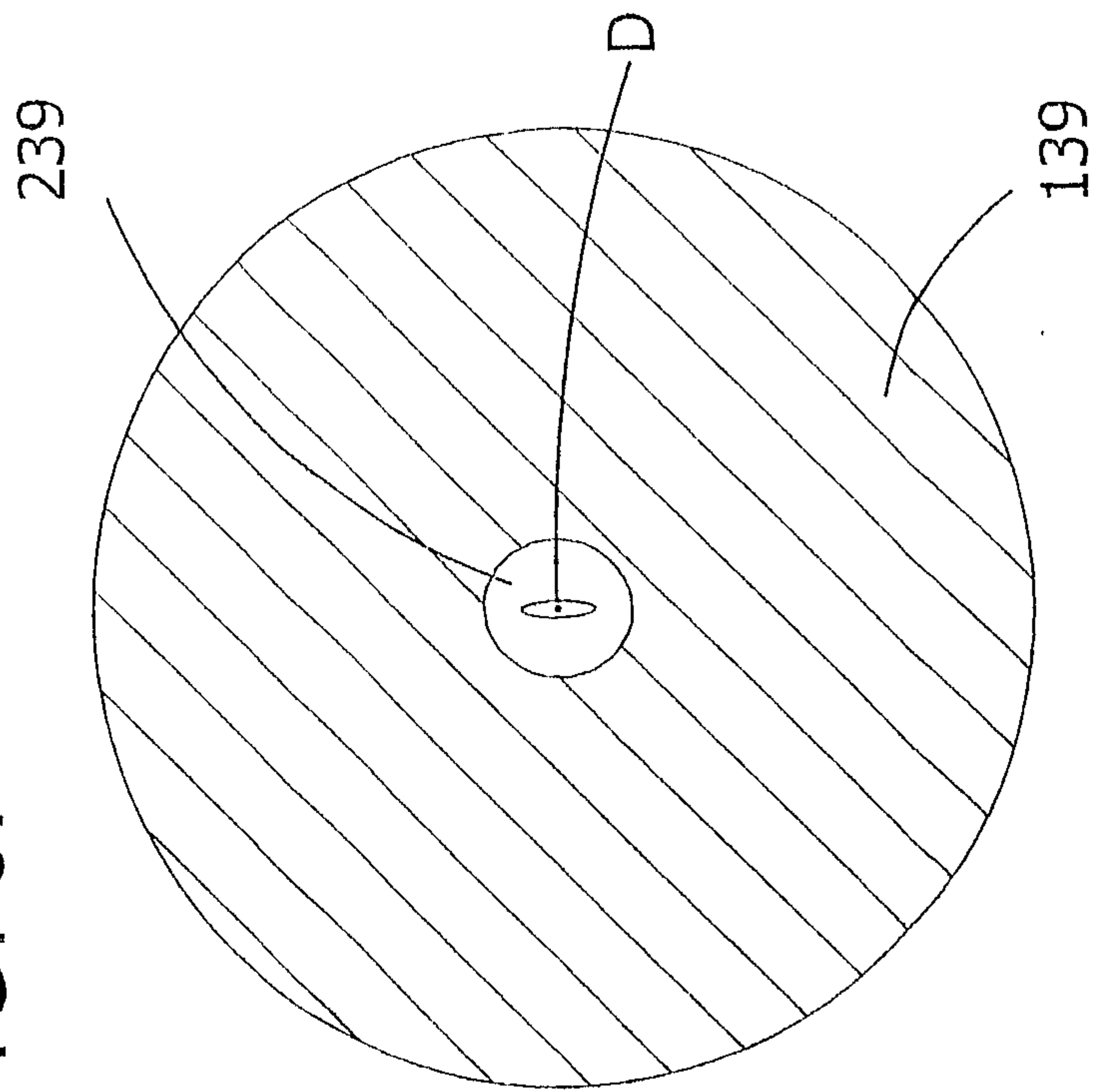


FIG. 9E

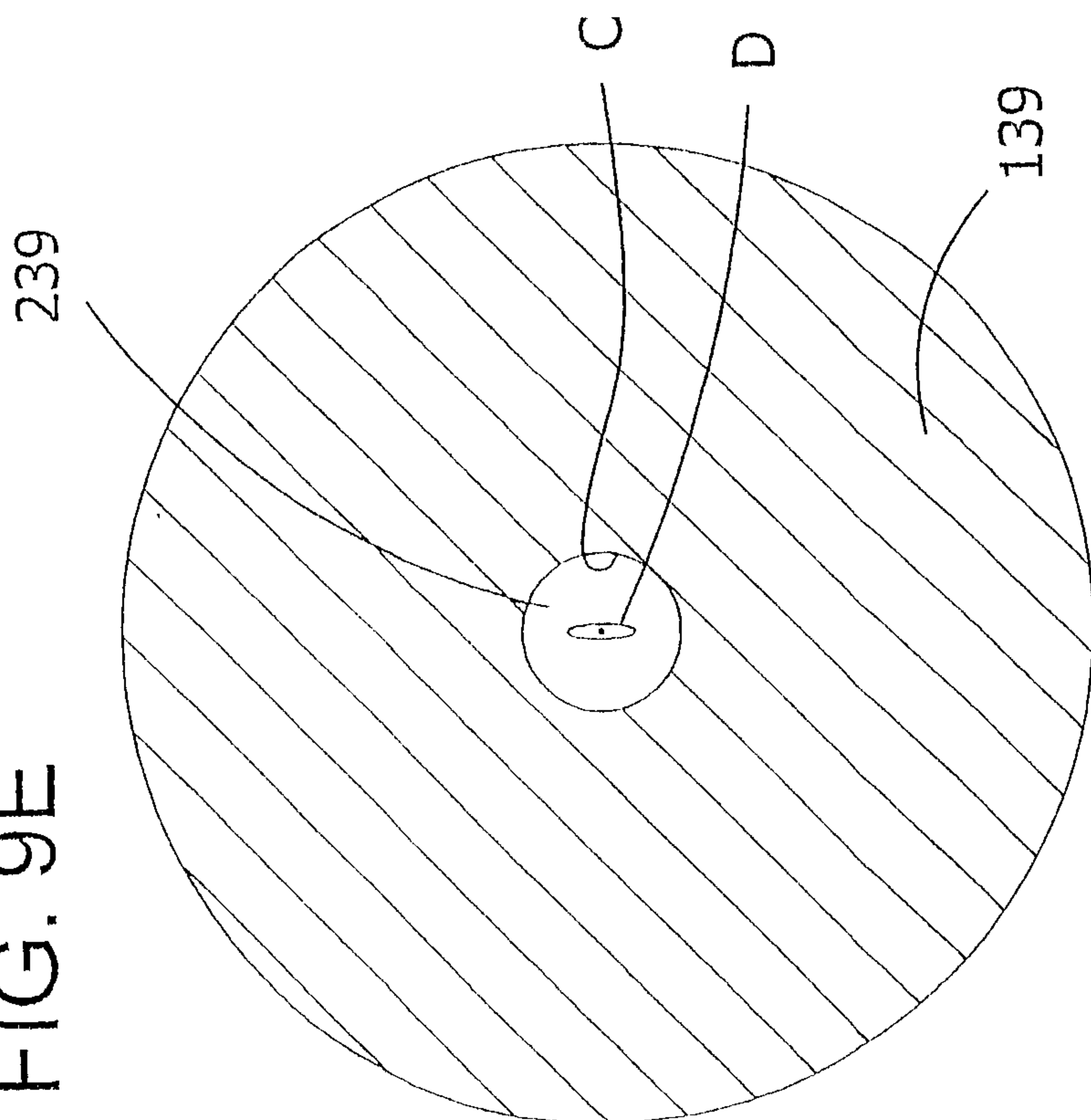


FIG. 9G

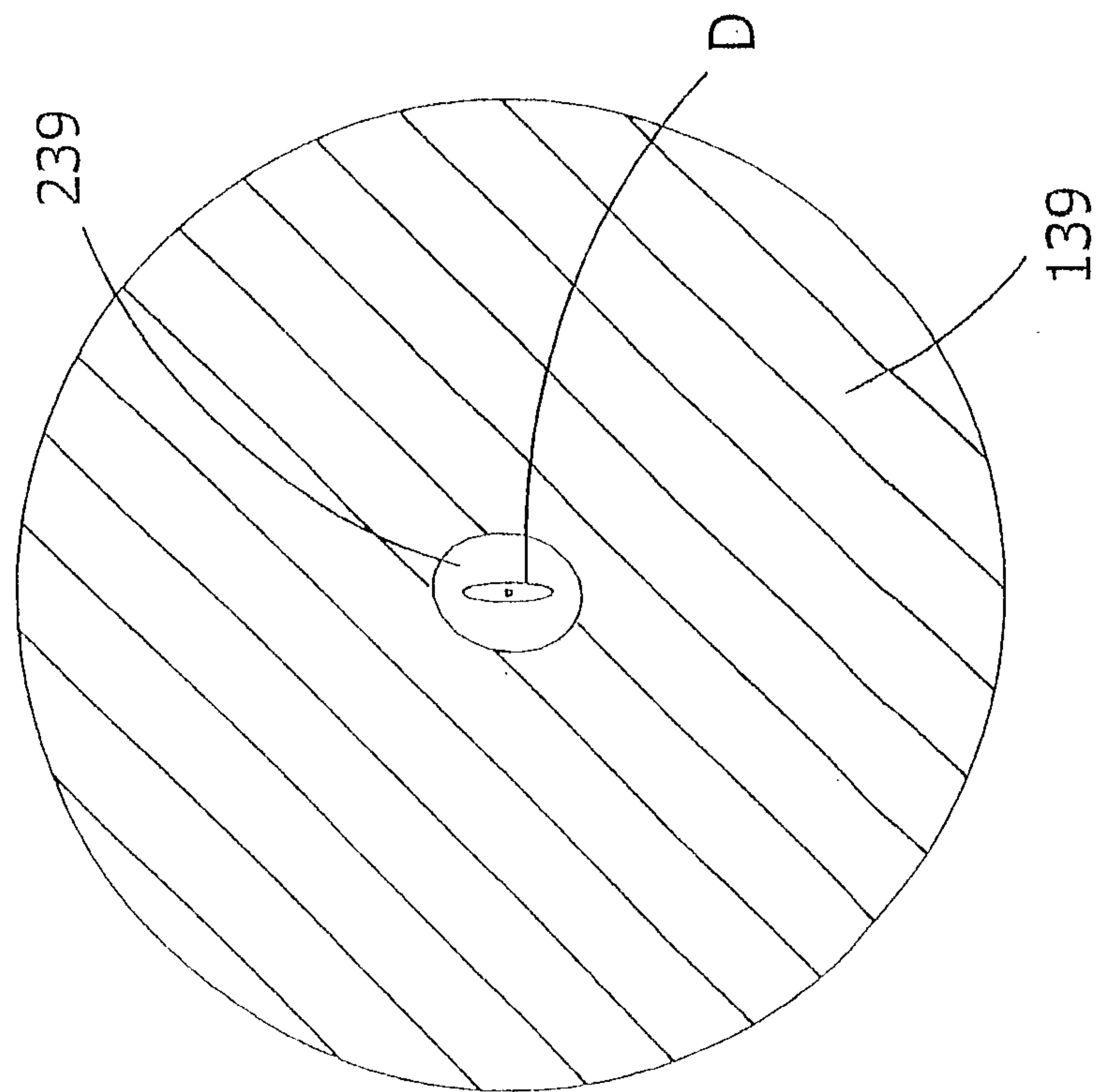


FIG. 9H

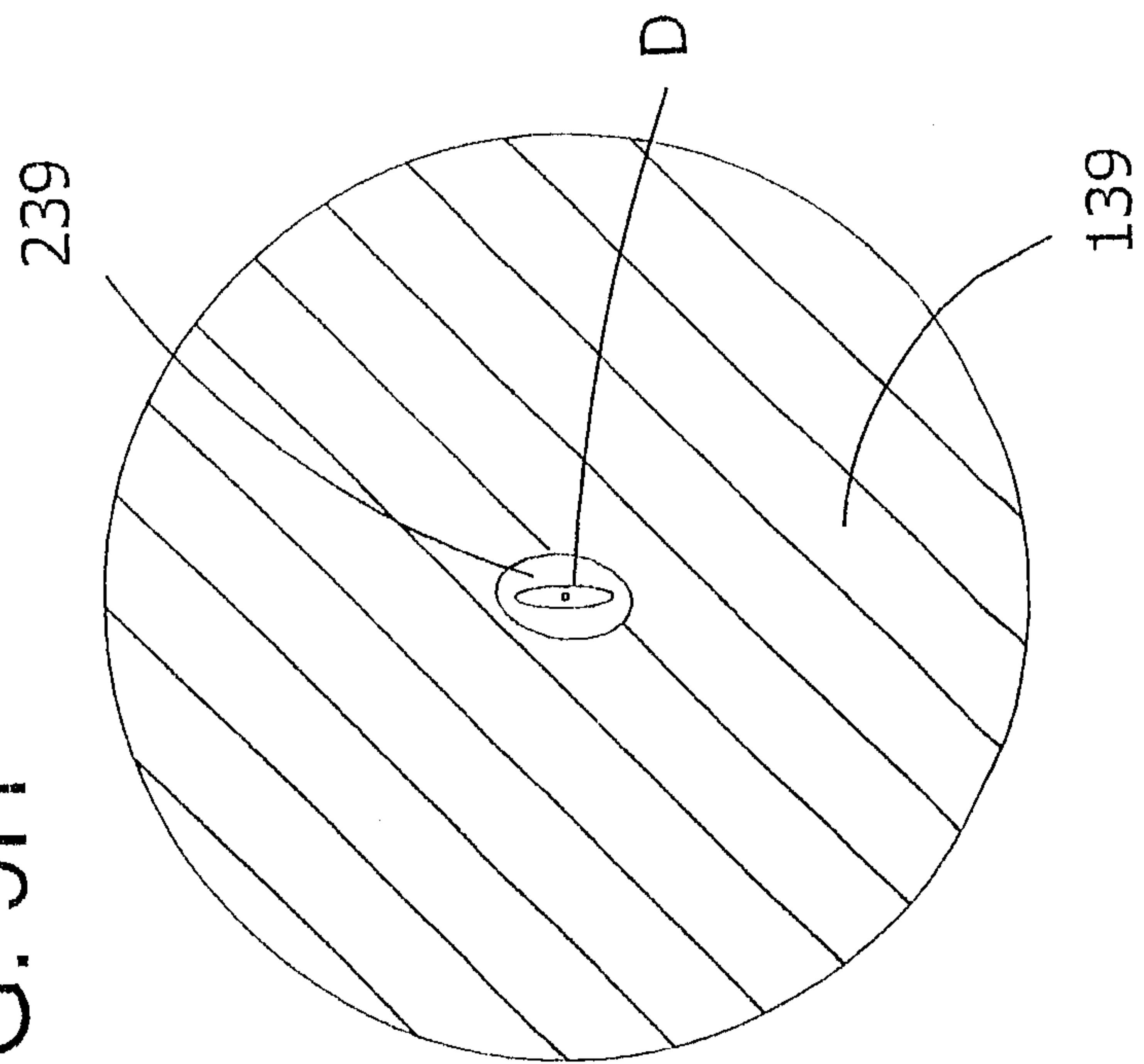


FIG. 9J

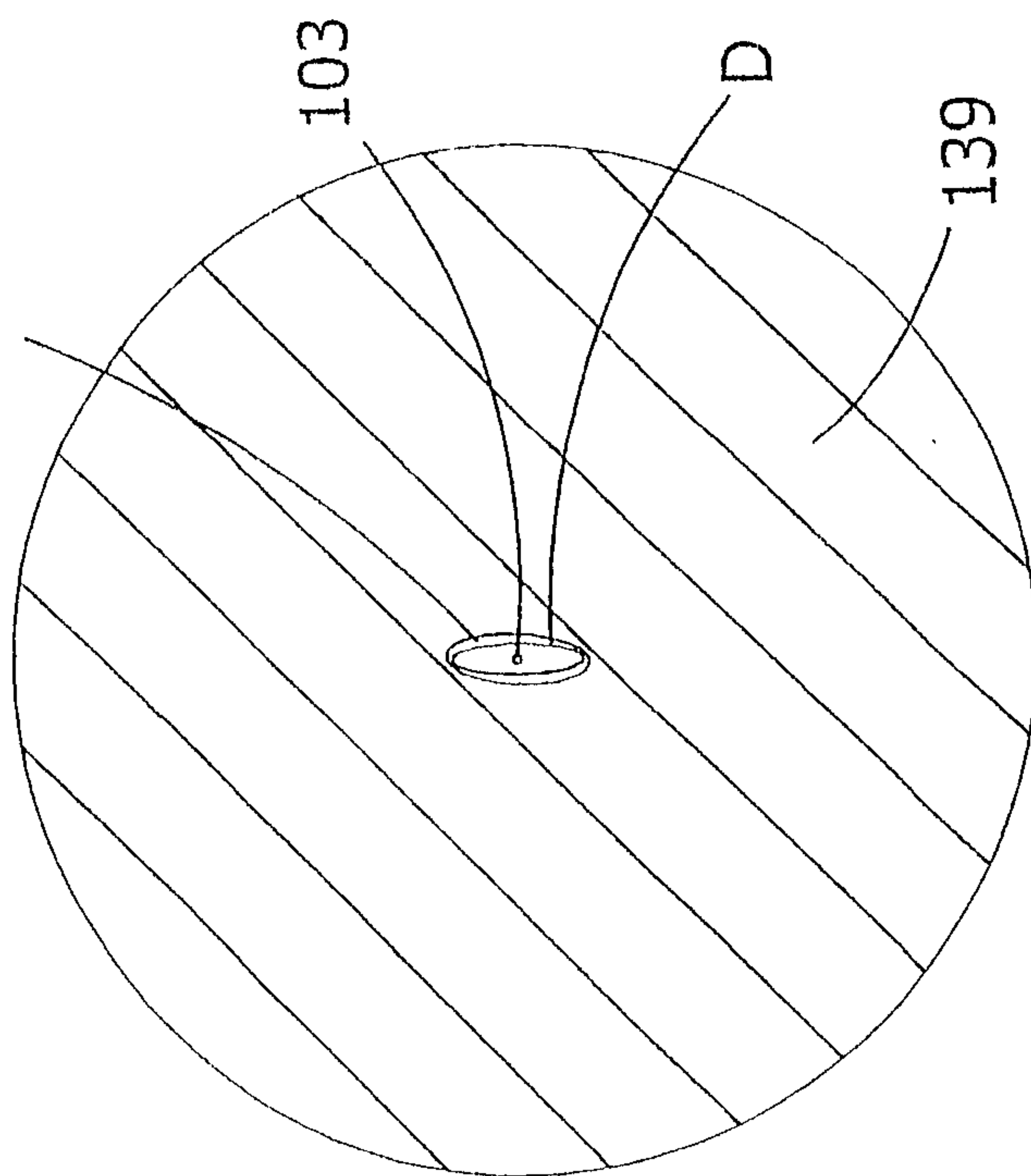
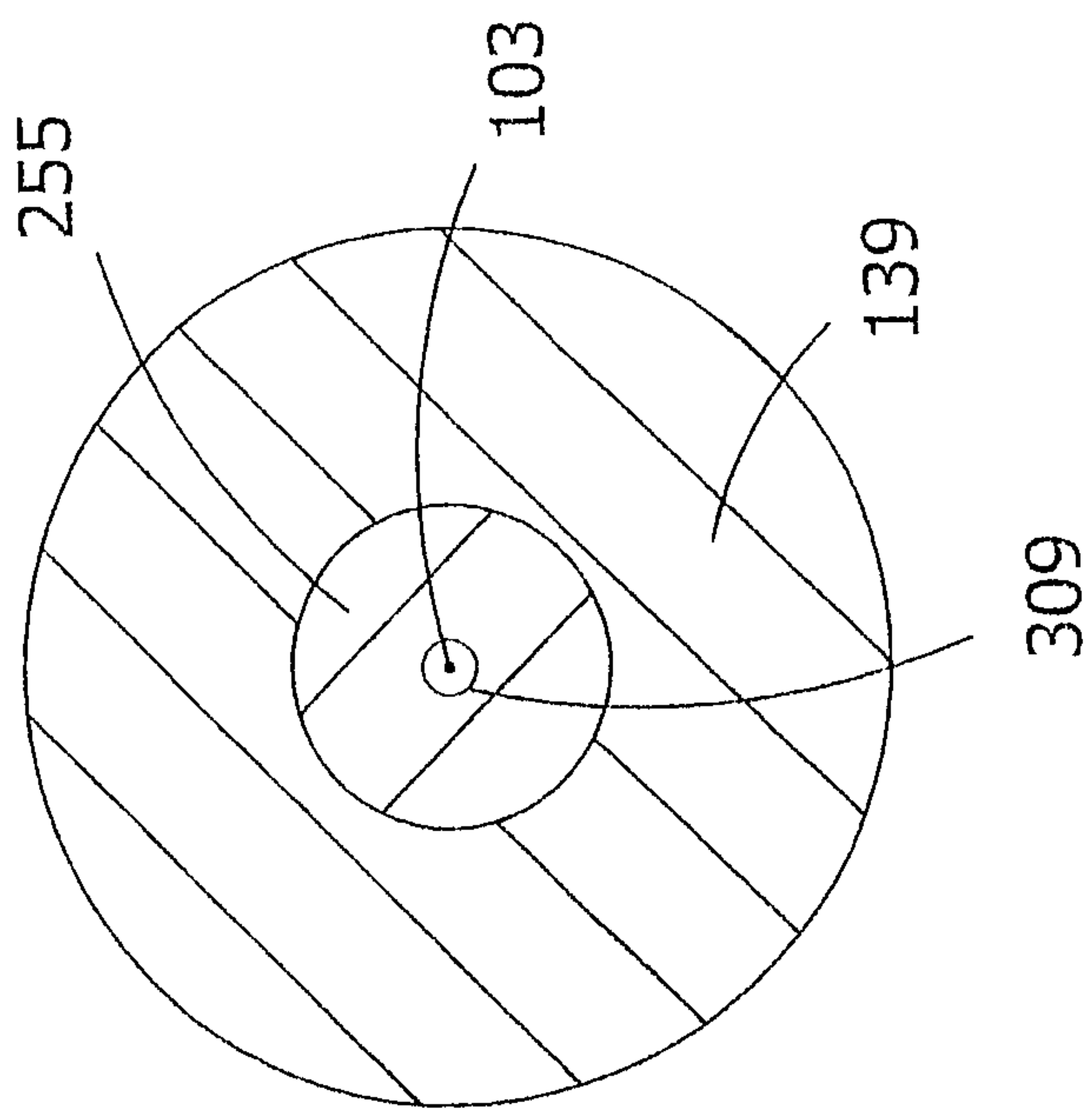
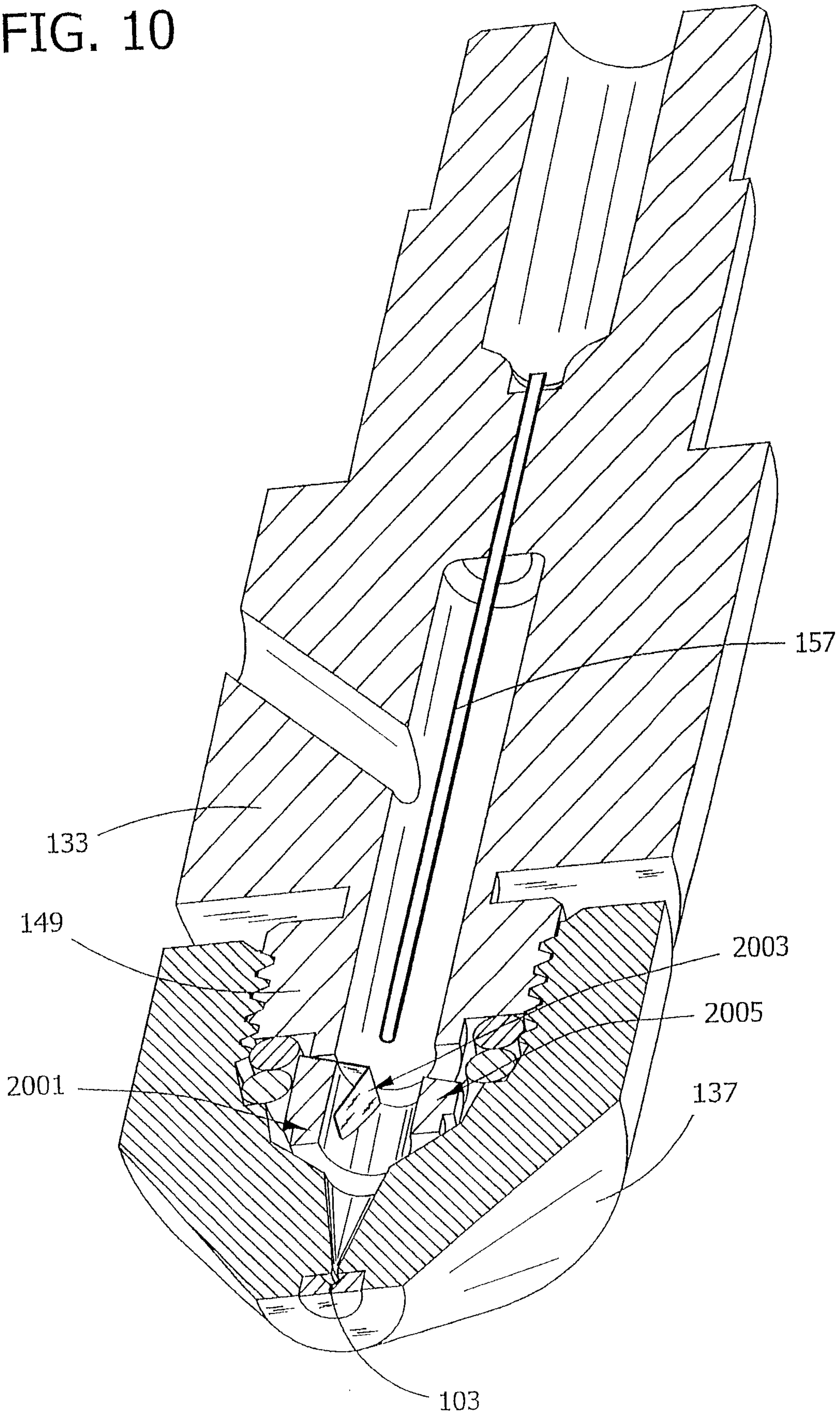


FIG. 9K



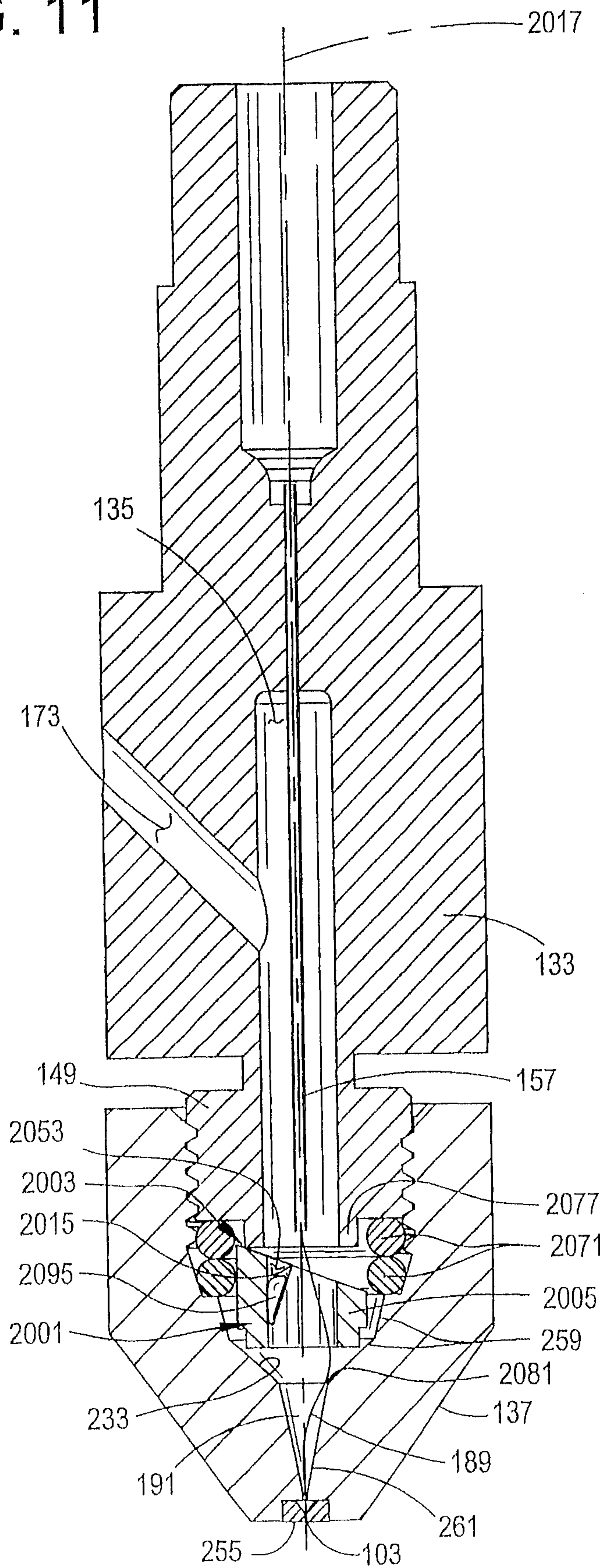
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FIG. 10



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FIG. 11



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FIG. 13

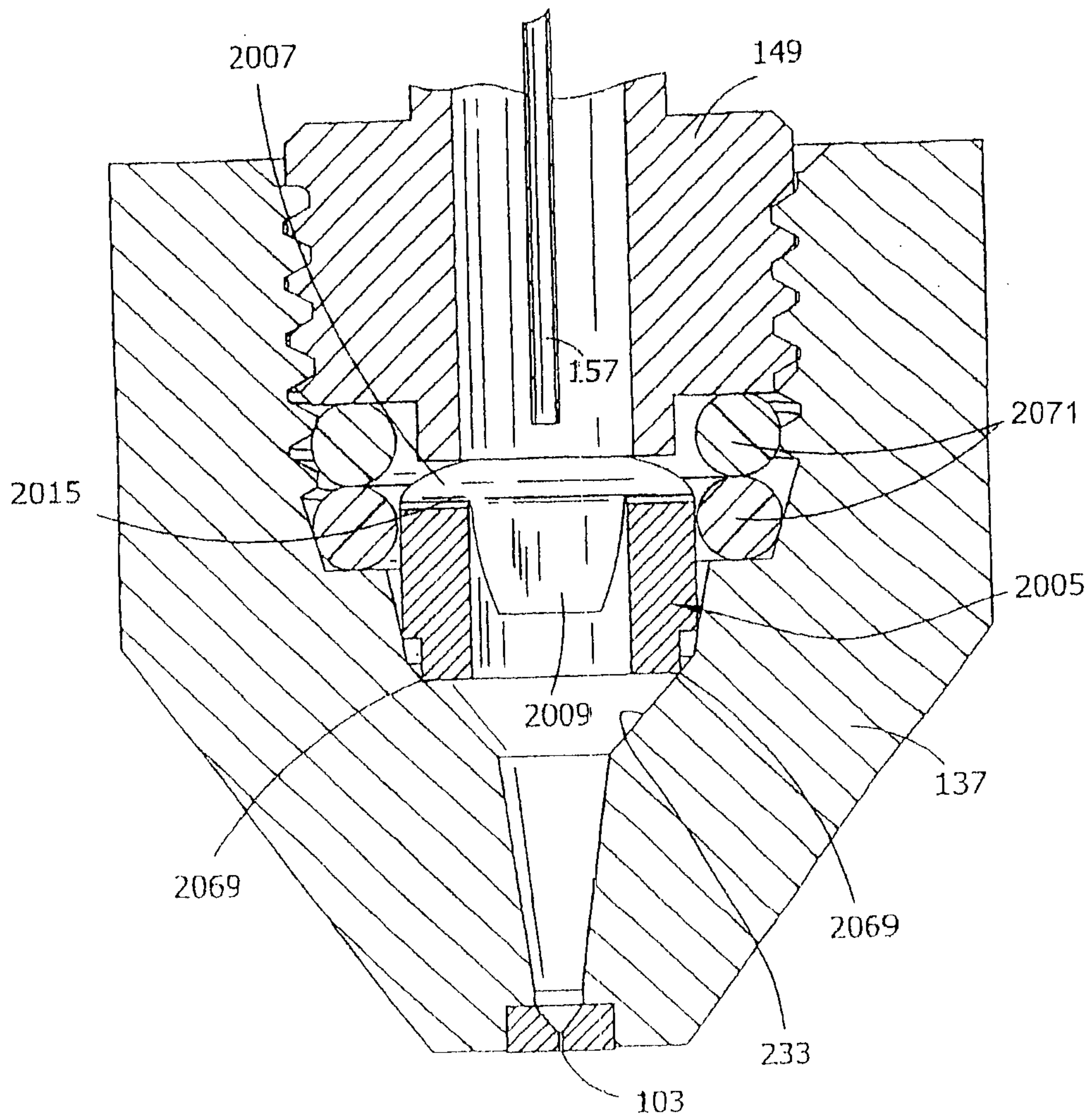
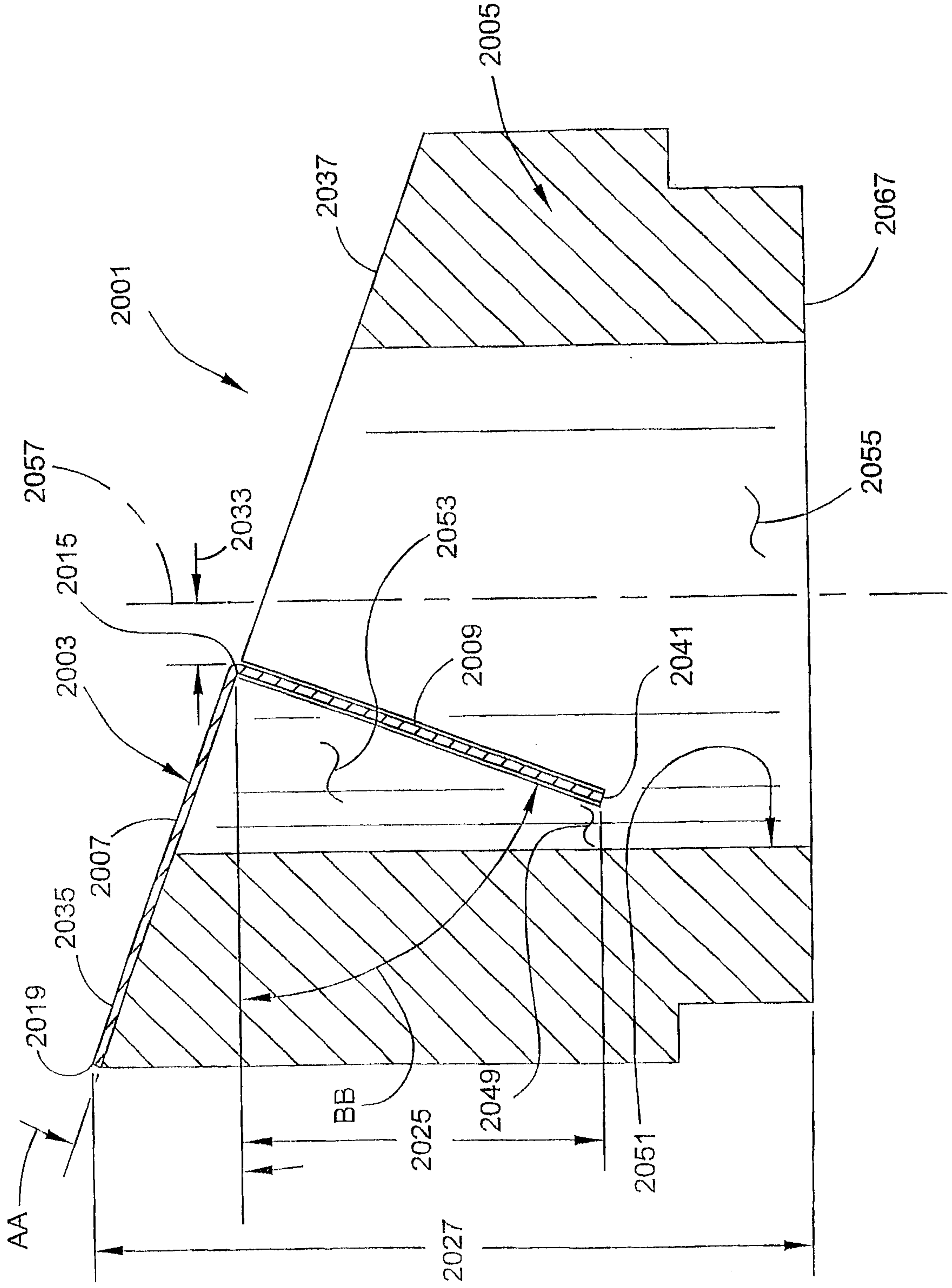
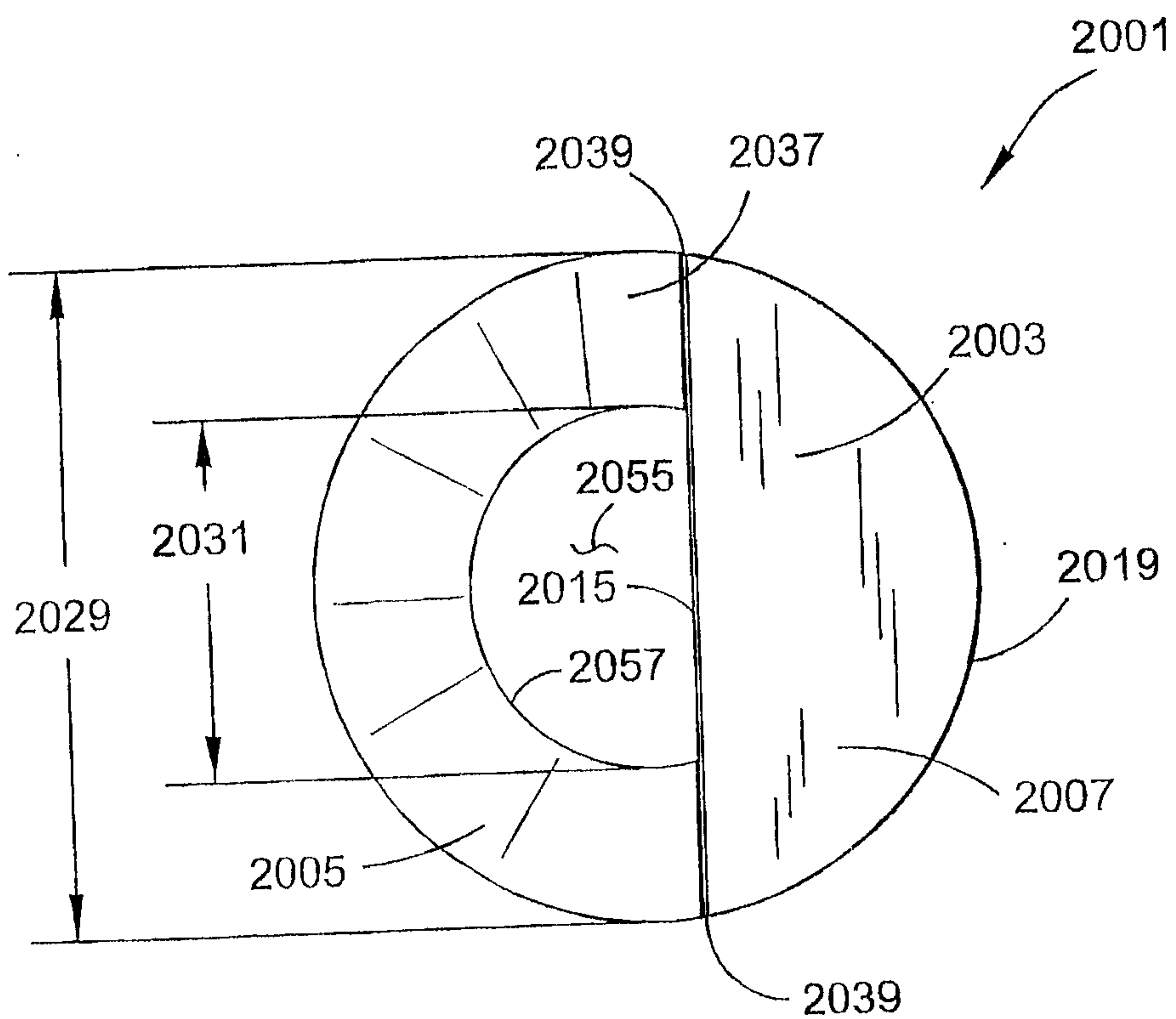


FIG. 14



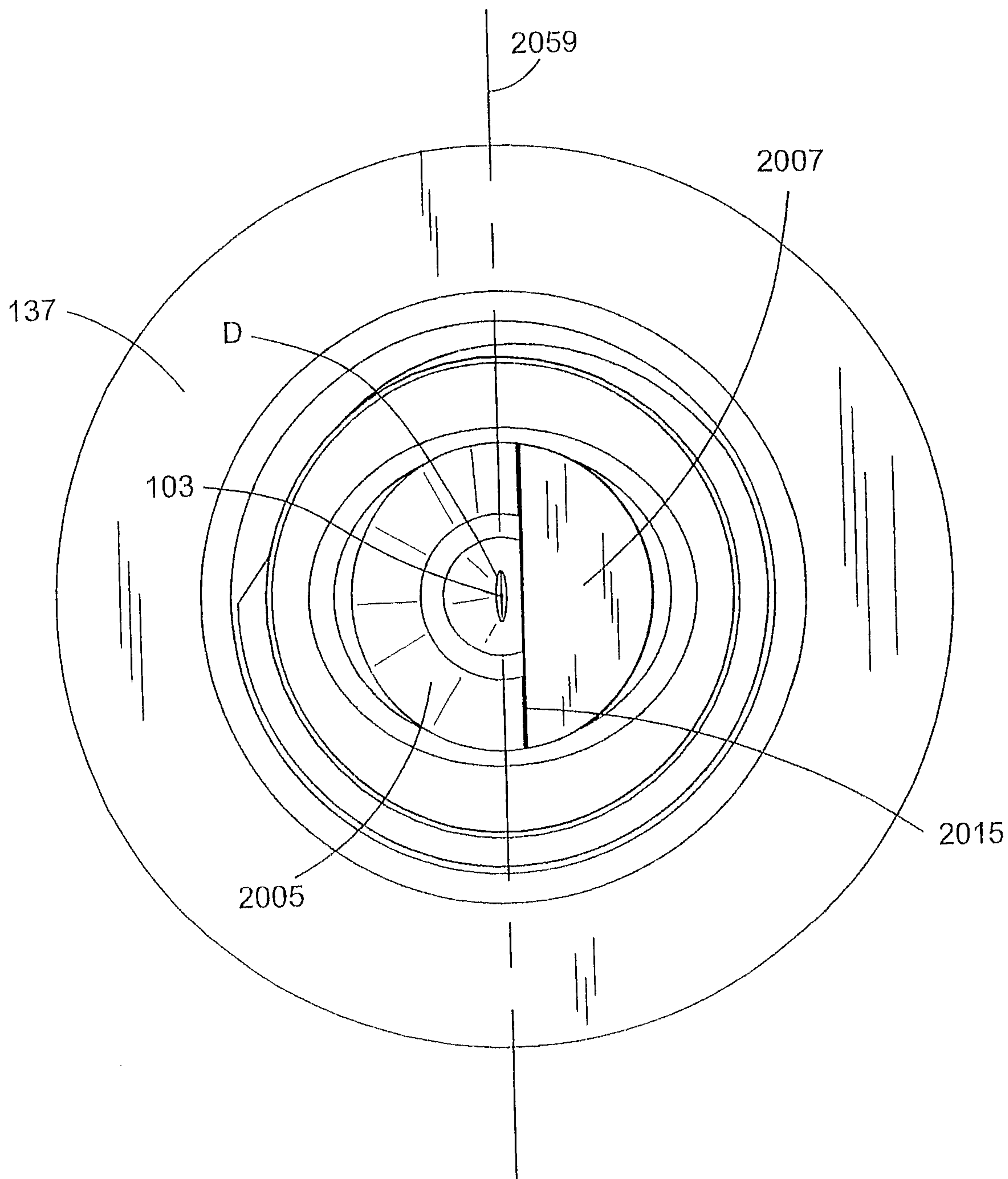
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FIG. 15



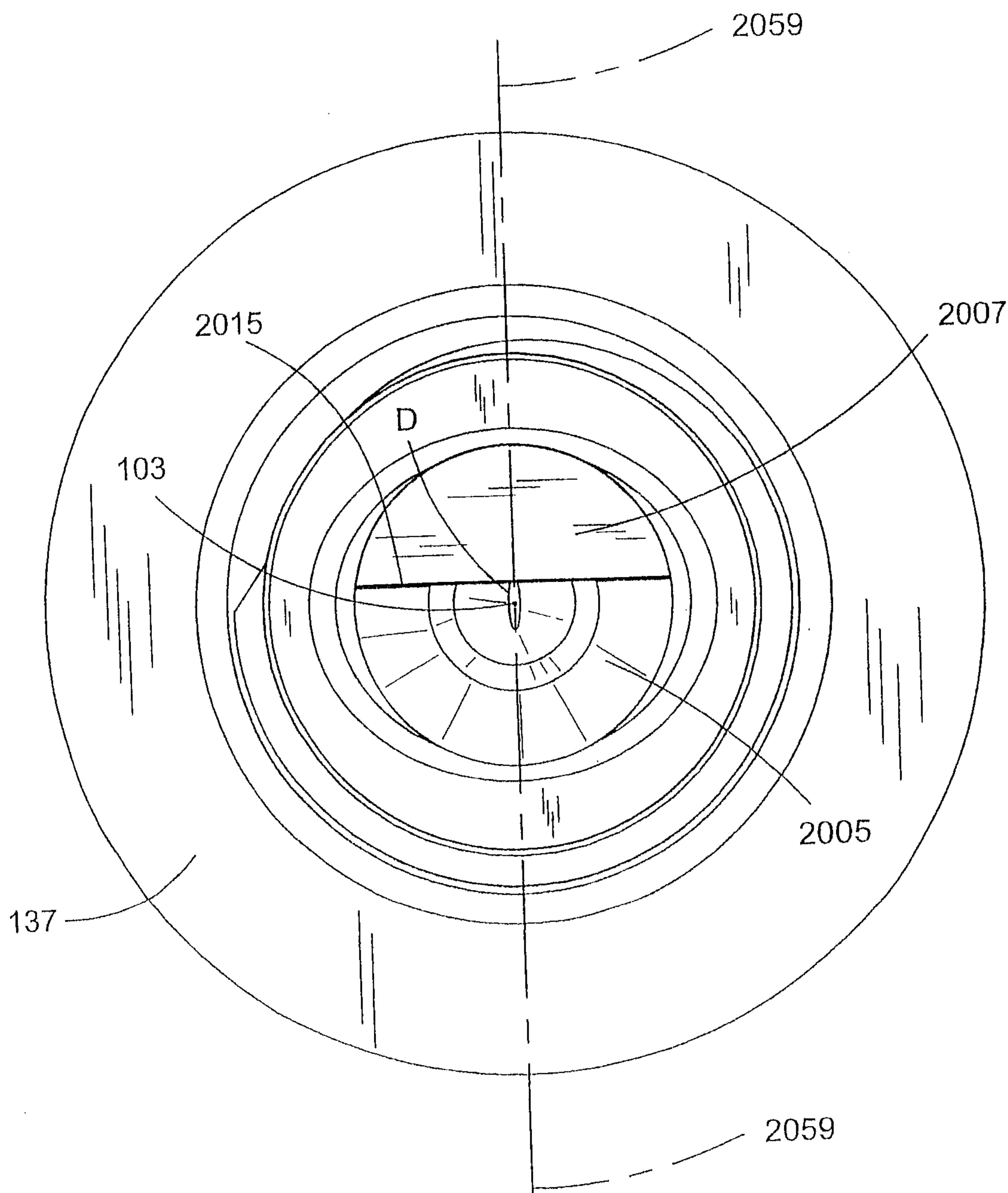
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FIG. 16

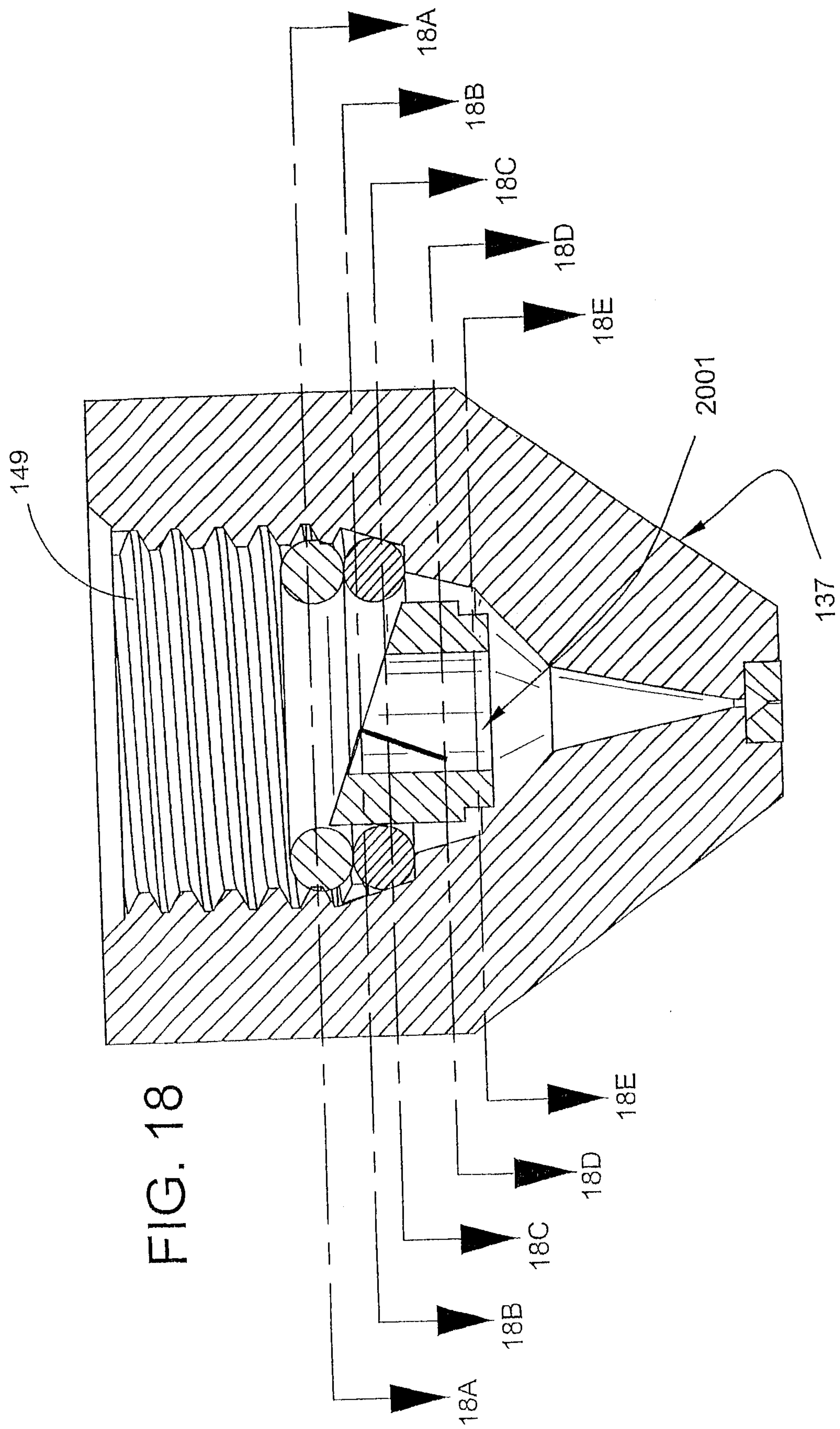


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FIG. 17



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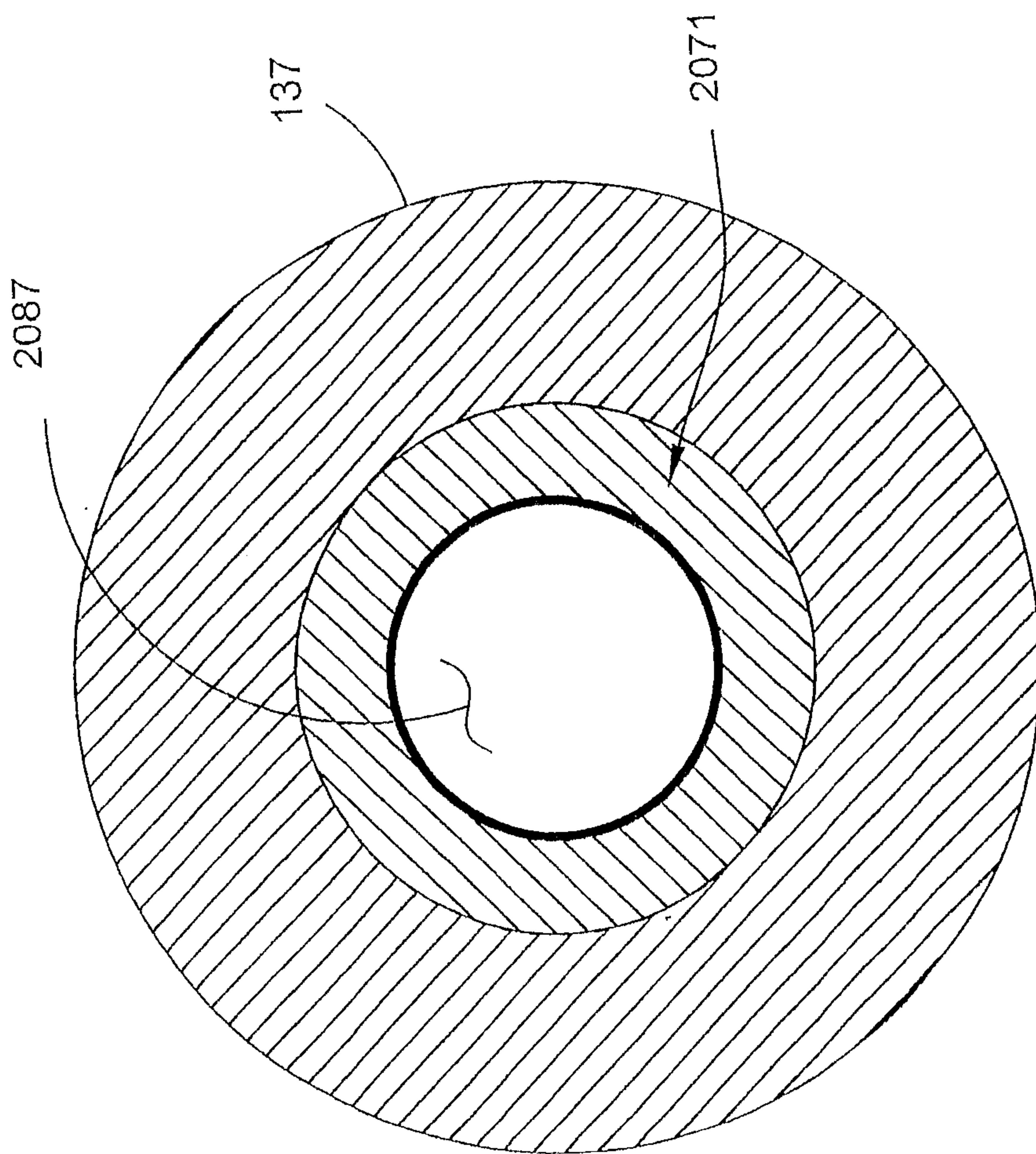


FIG. 18A

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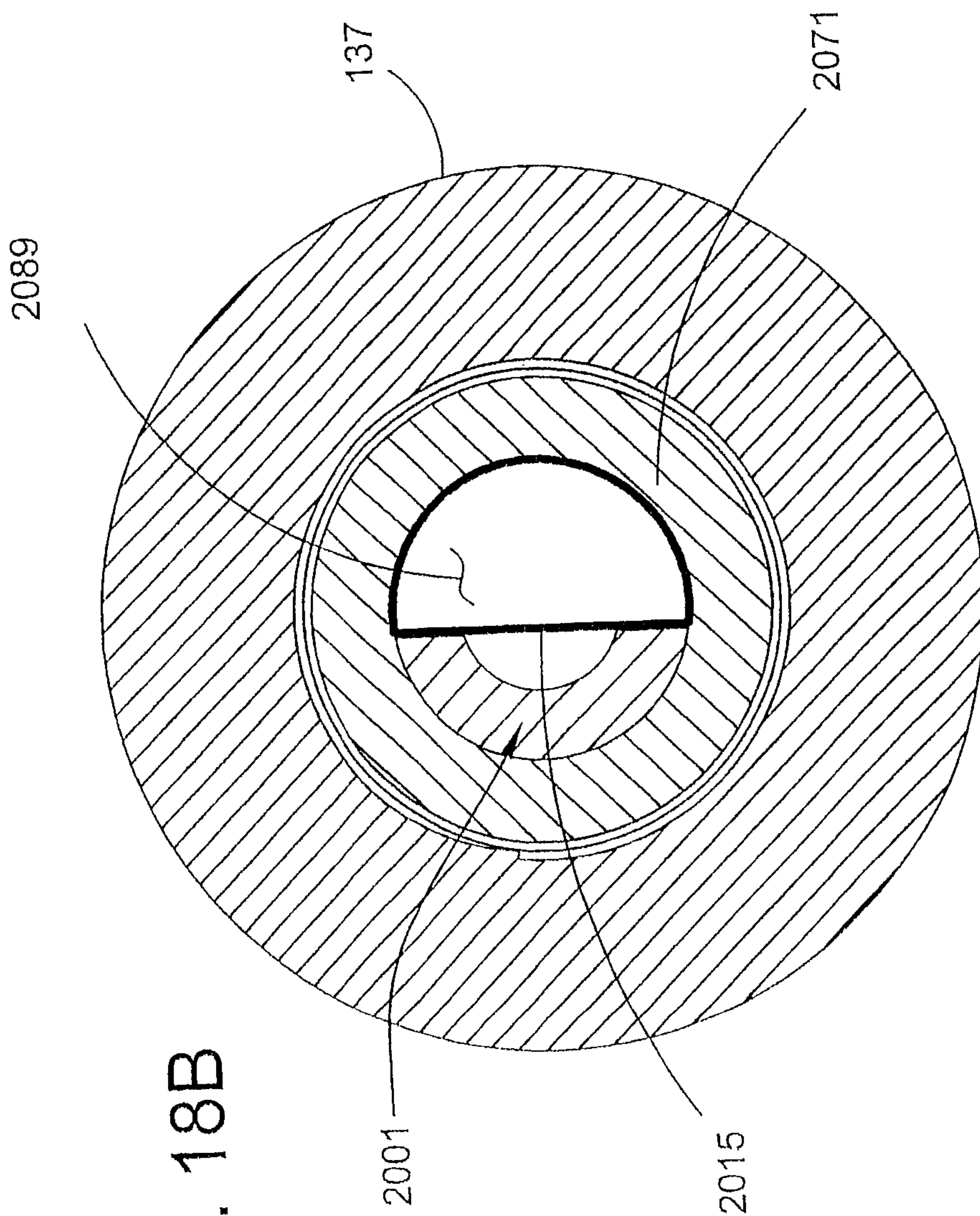


FIG. 18B

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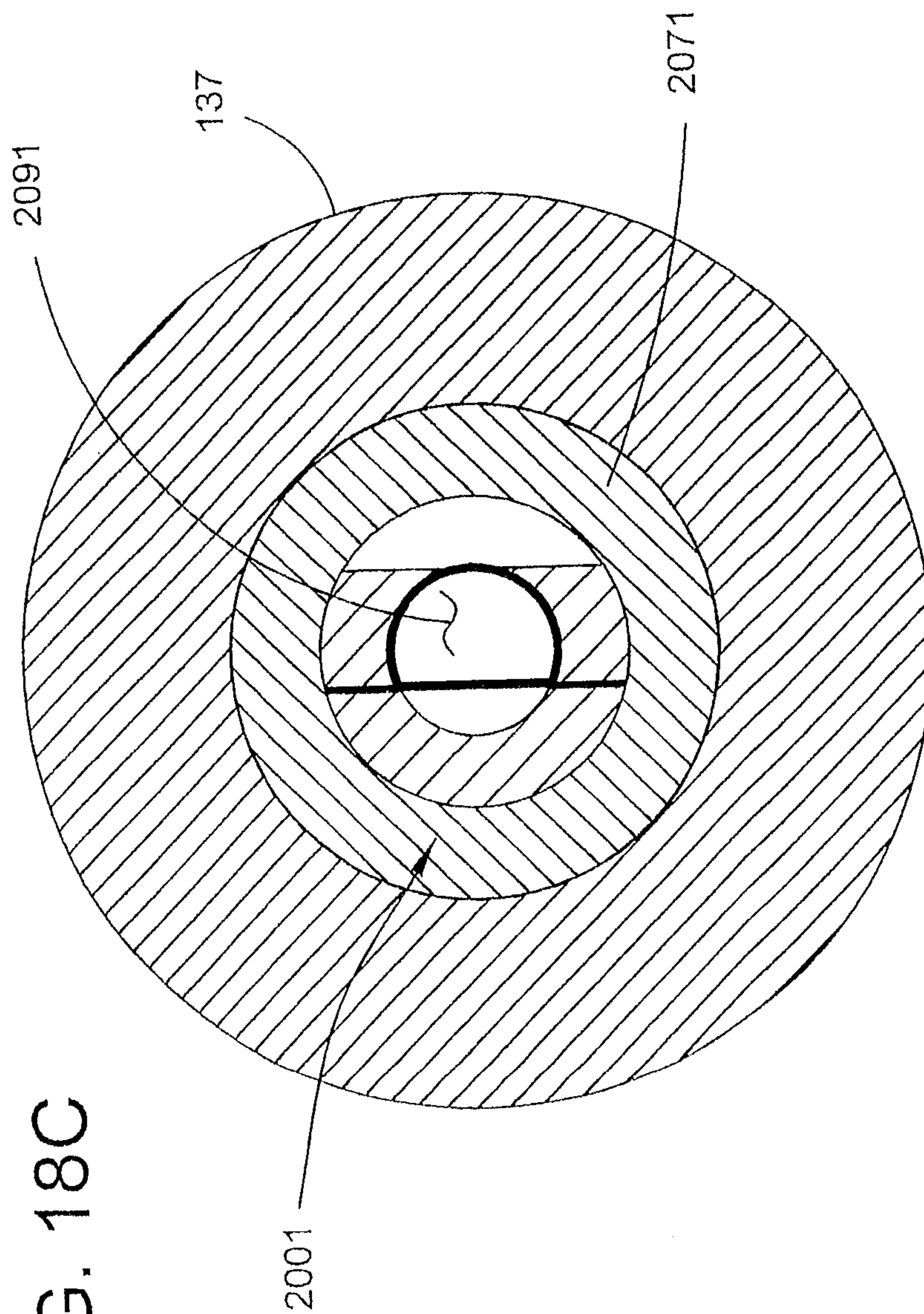


FIG. 18C

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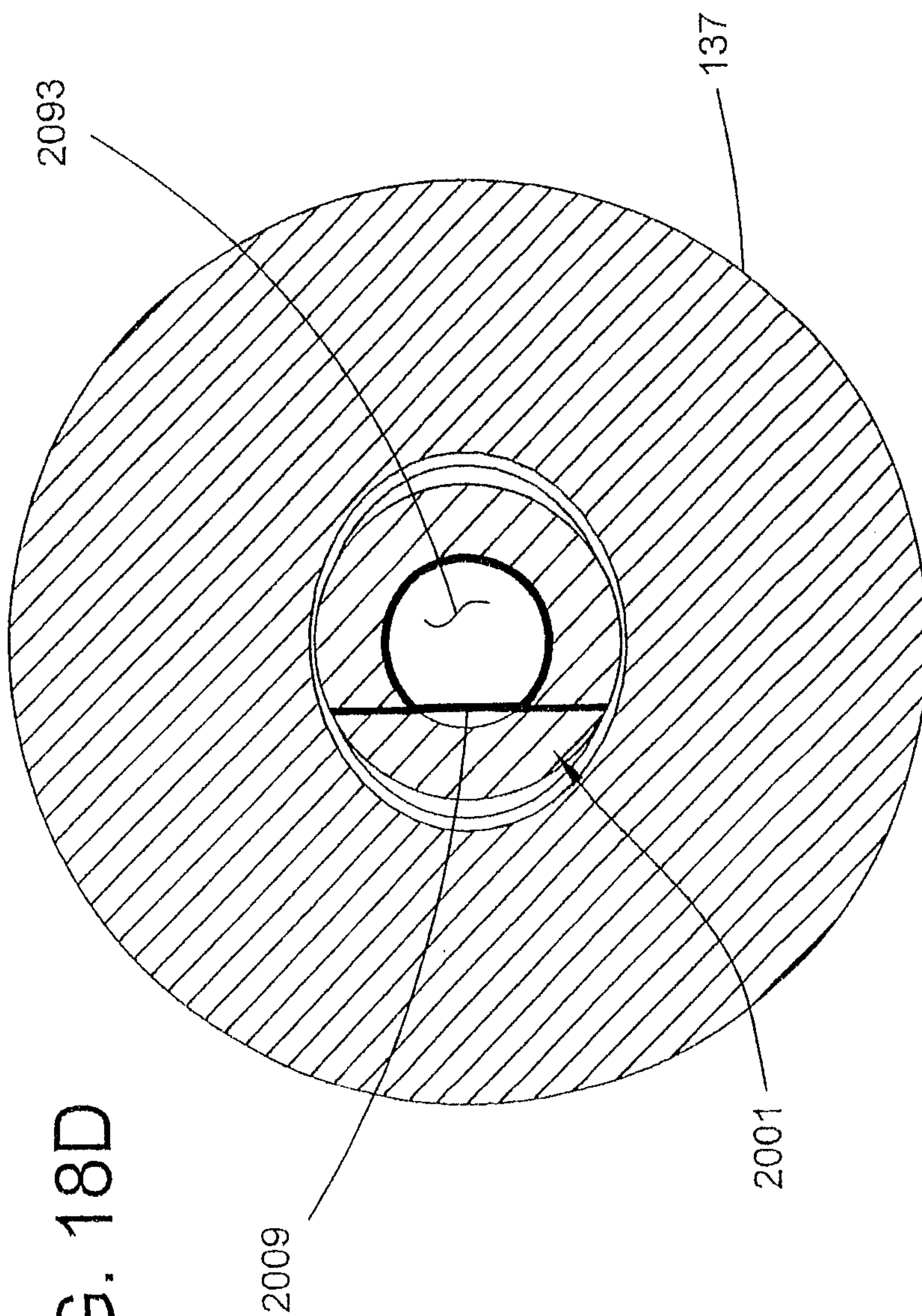


FIG. 18D

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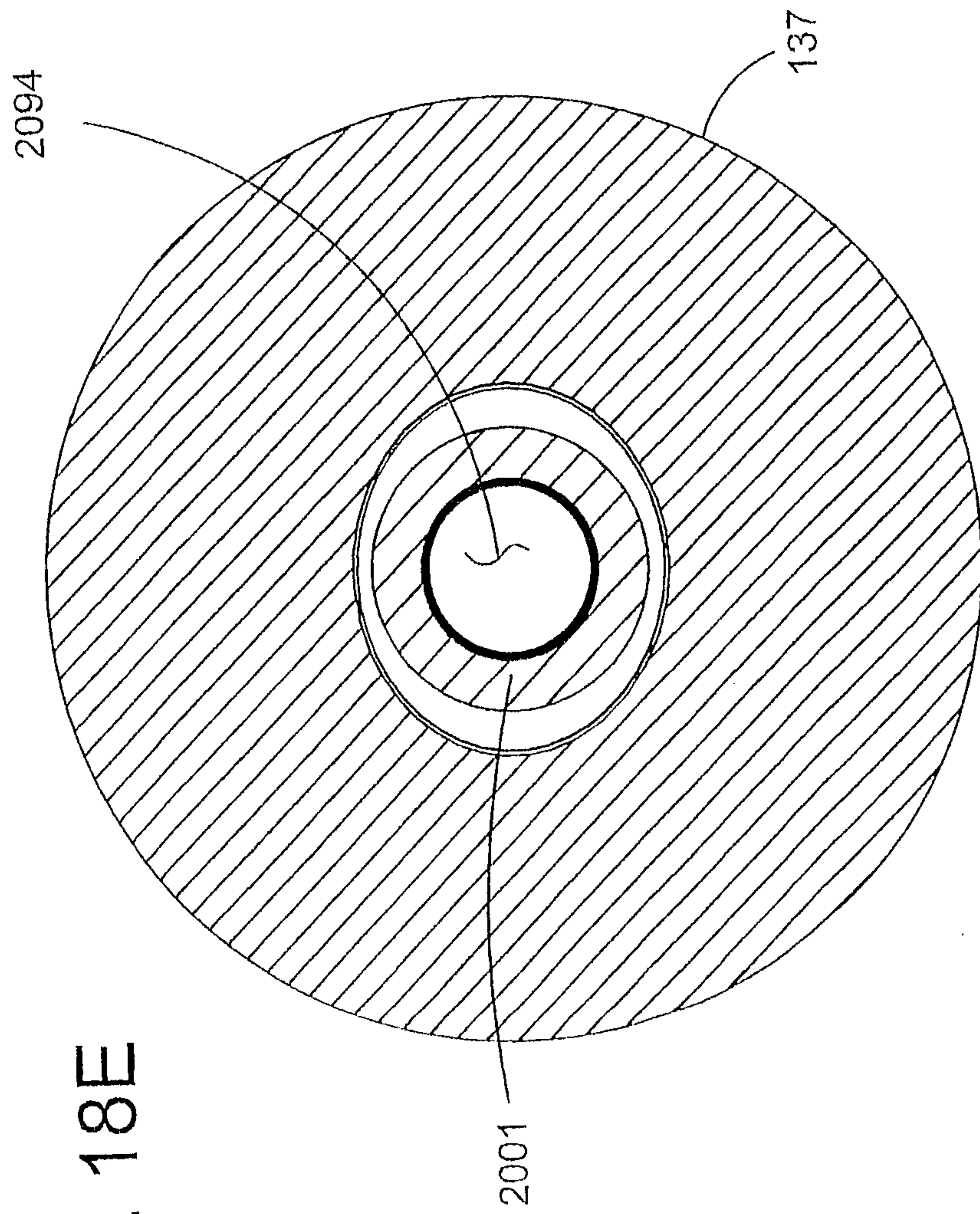
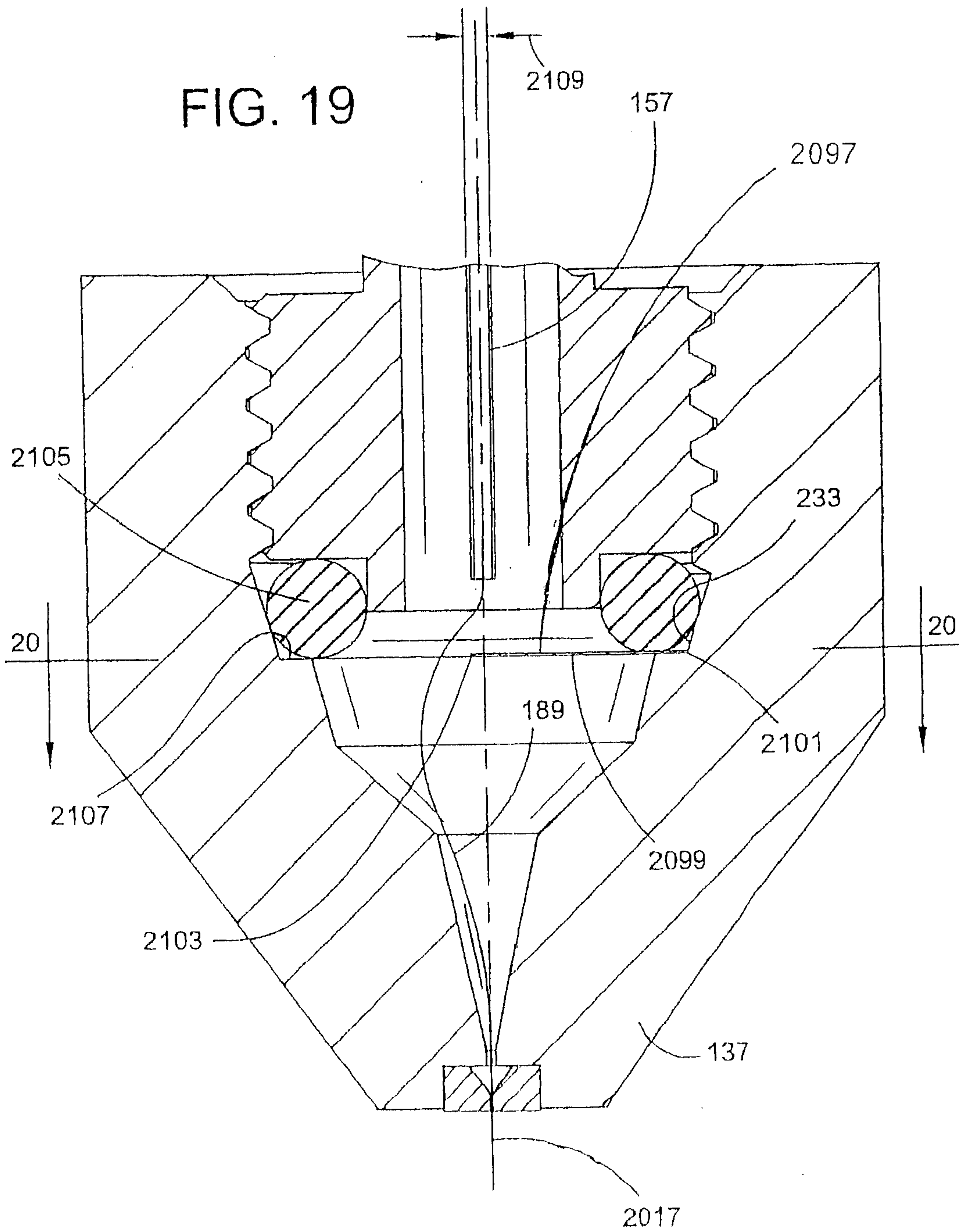


FIG. 18E

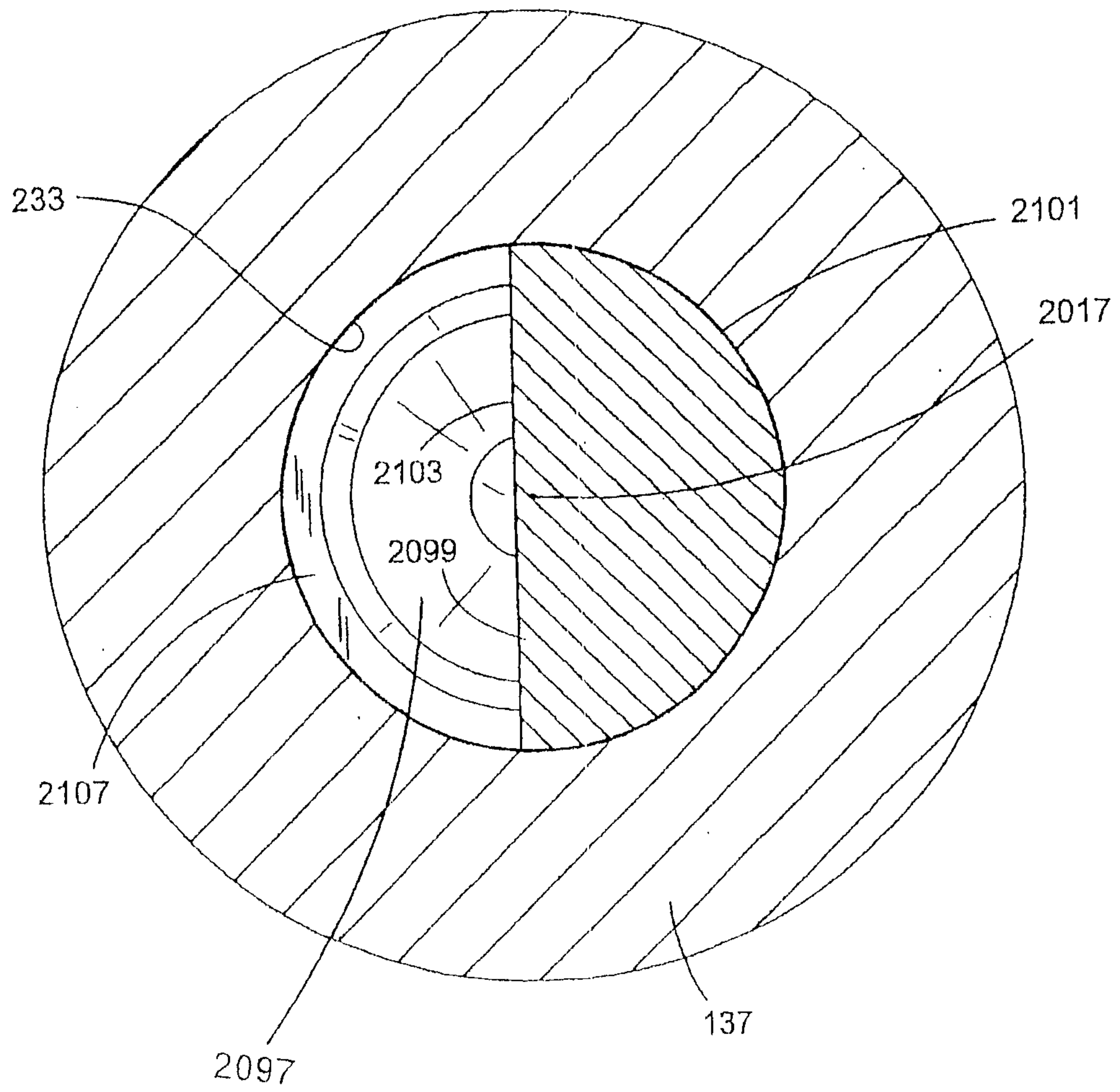
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FIG. 19



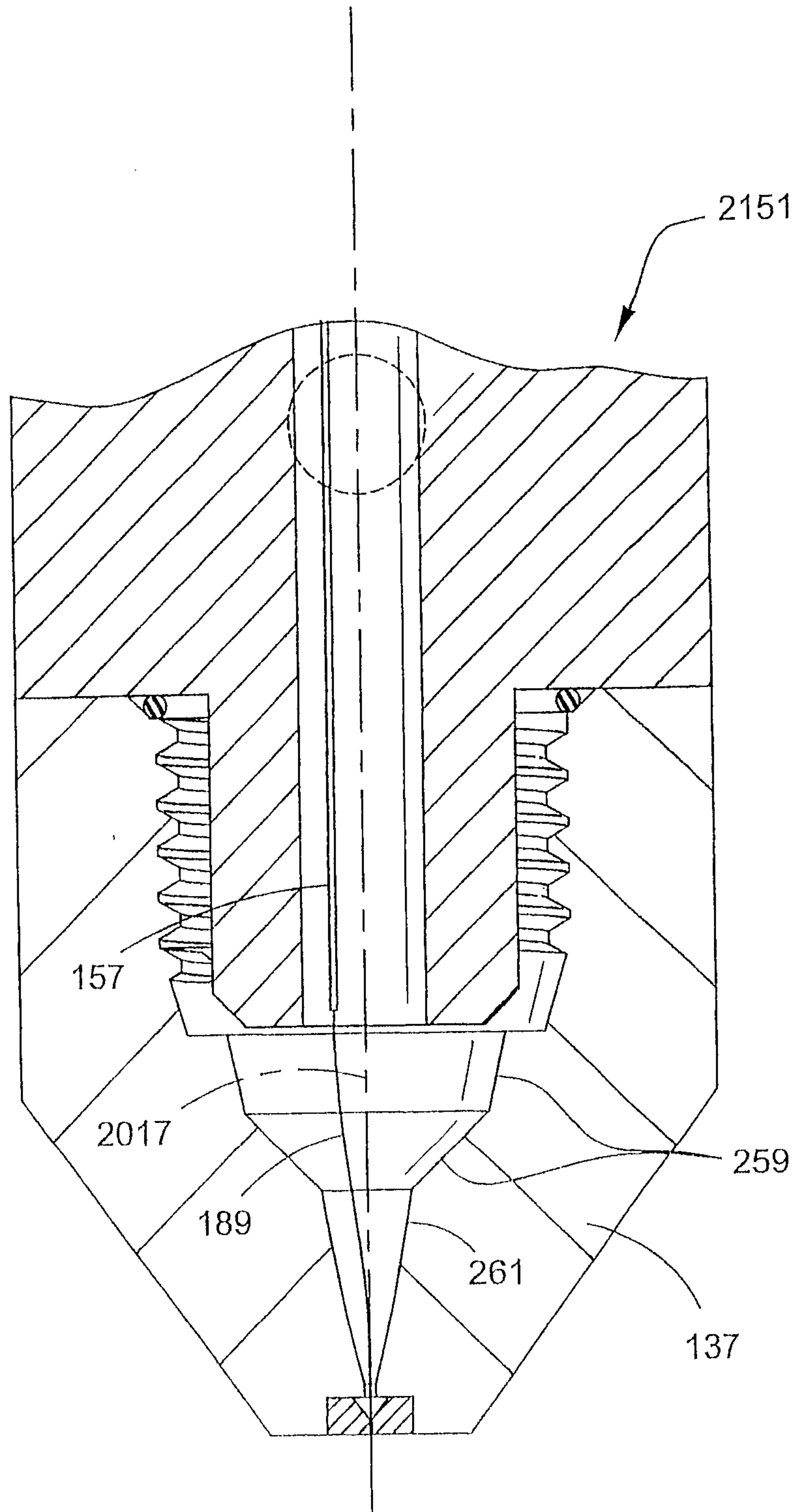
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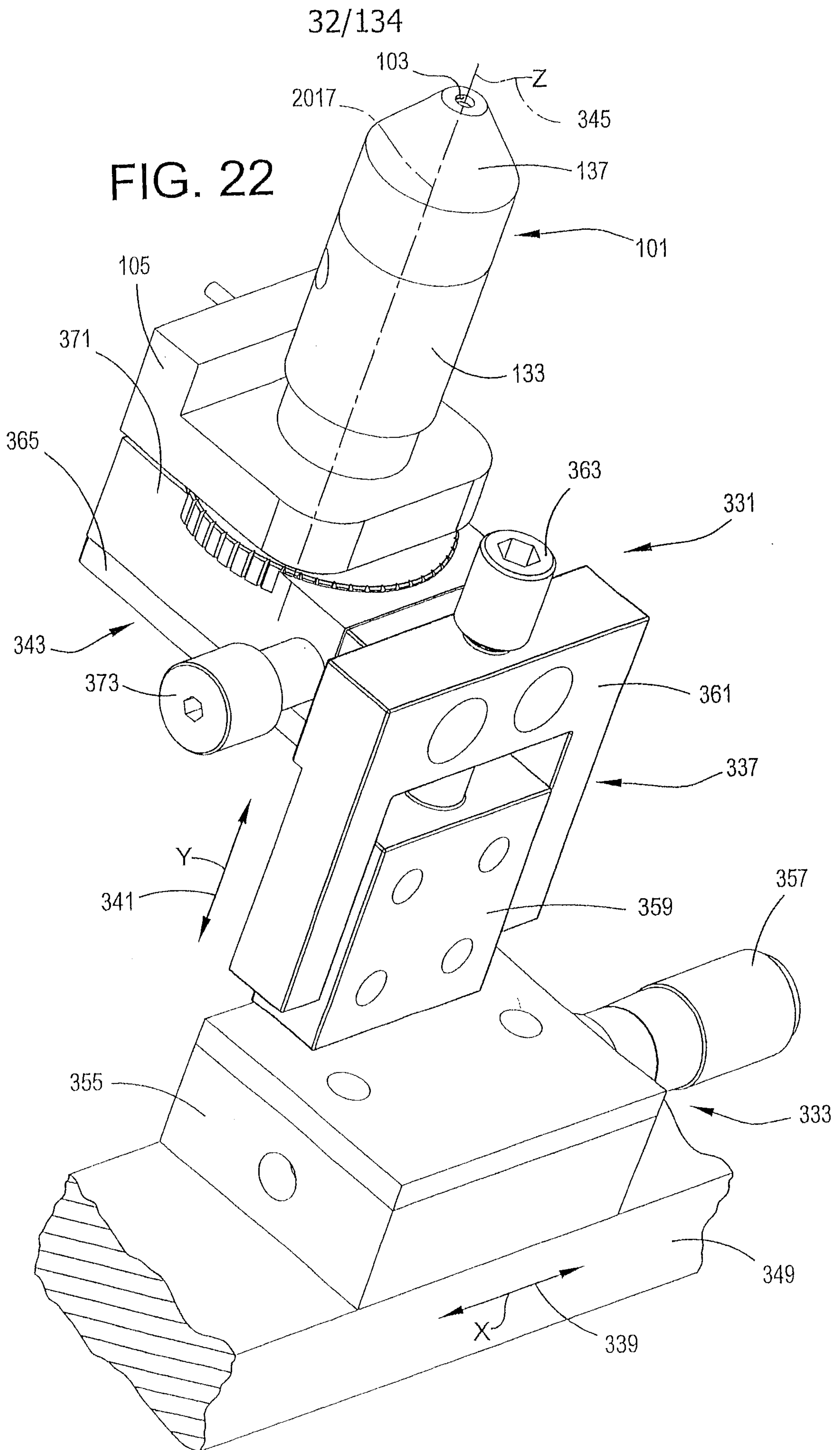
FIG. 20



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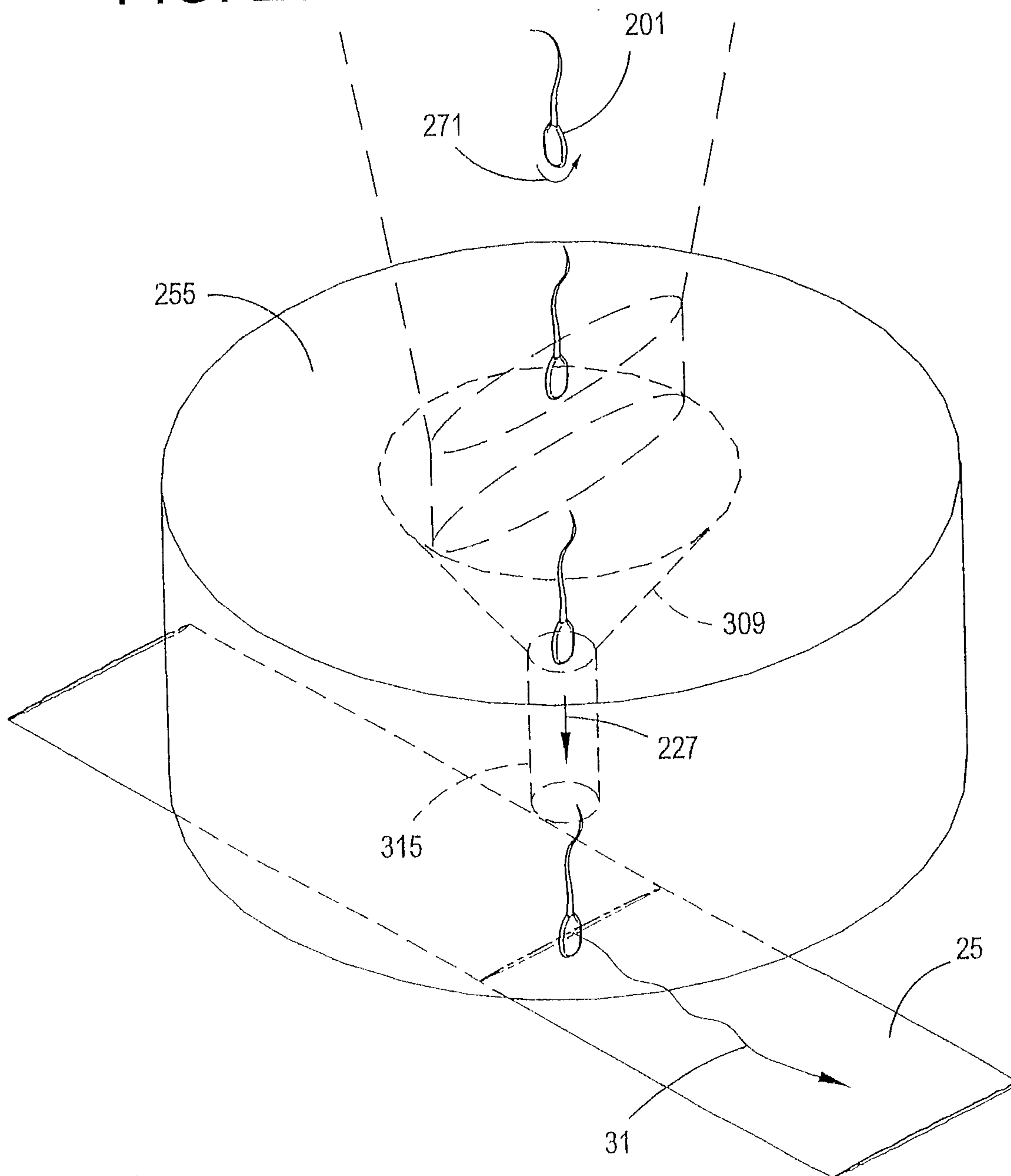
FIG. 21

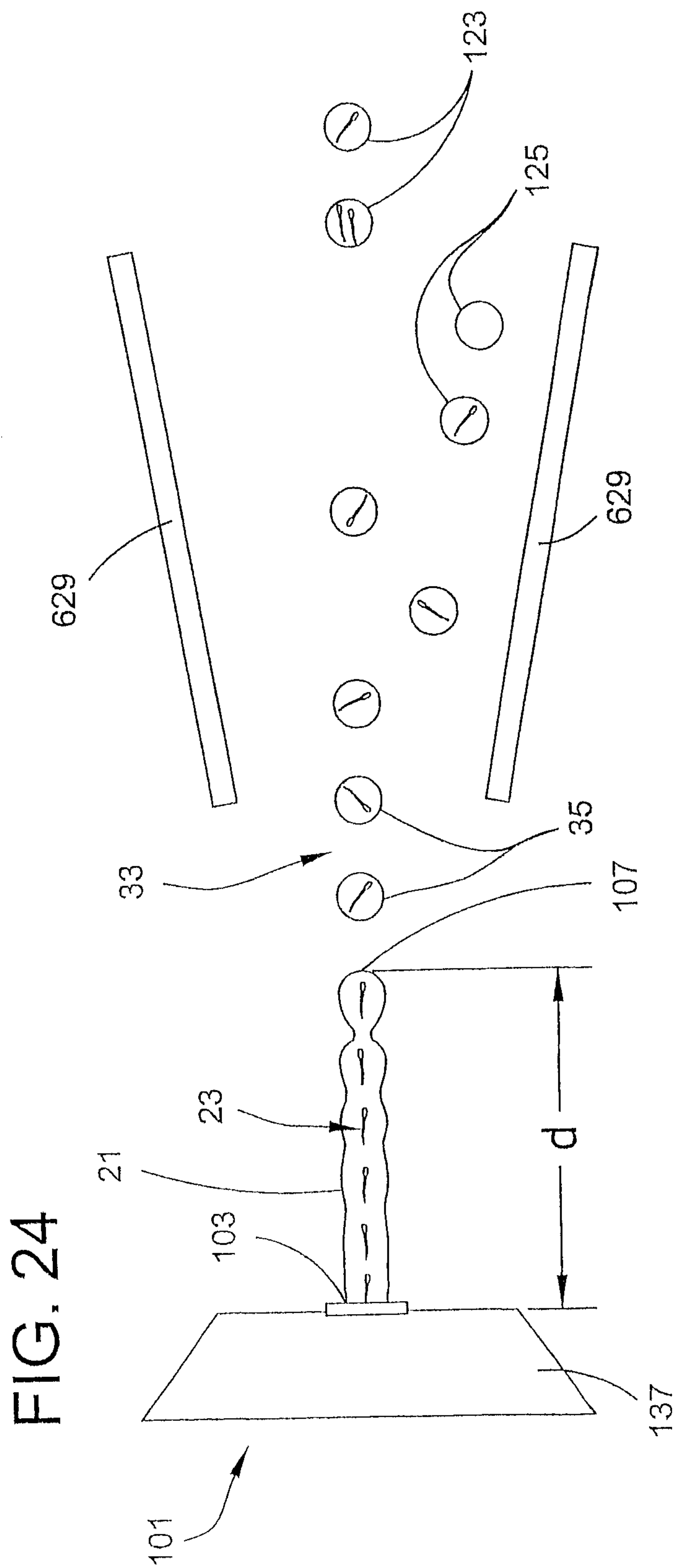




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FIG. 23





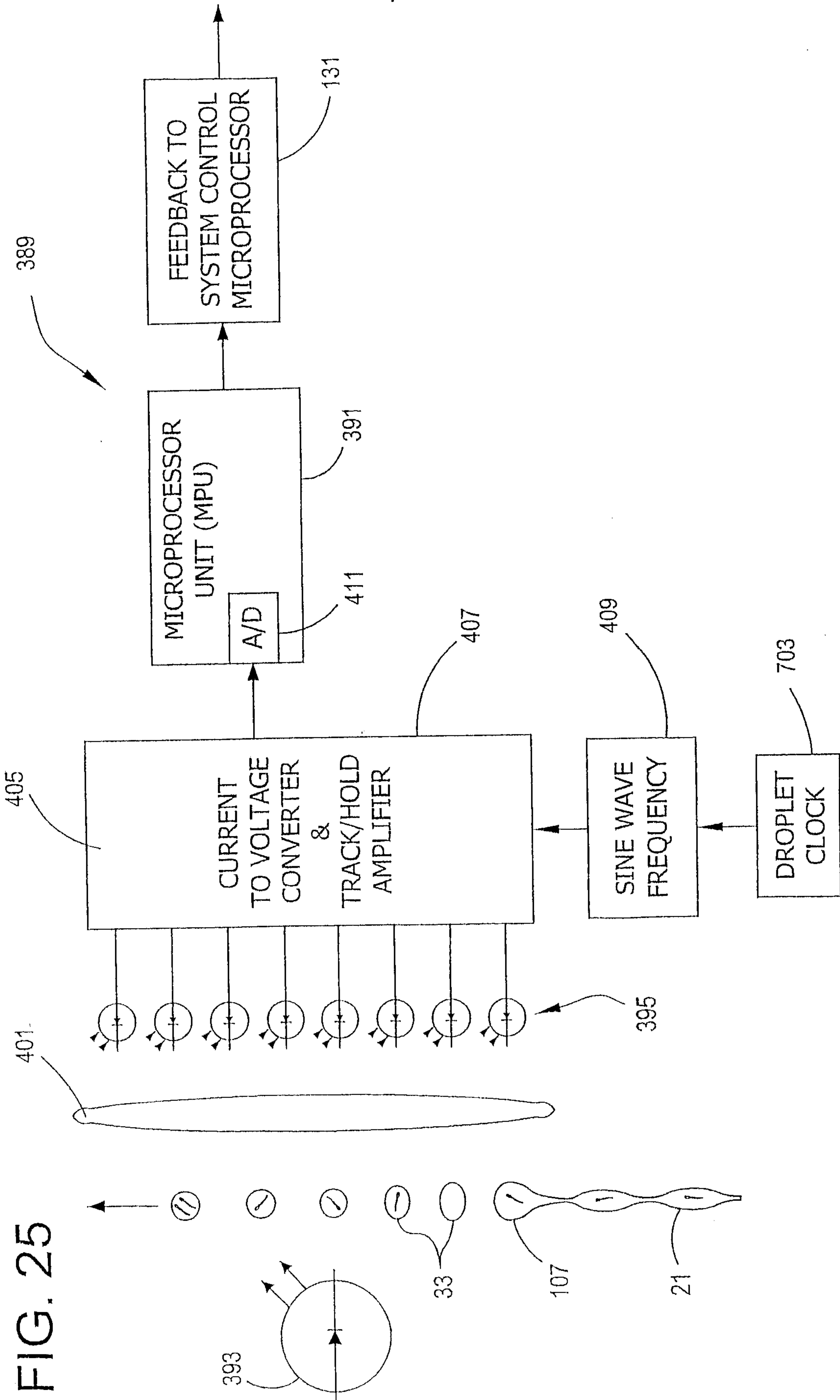
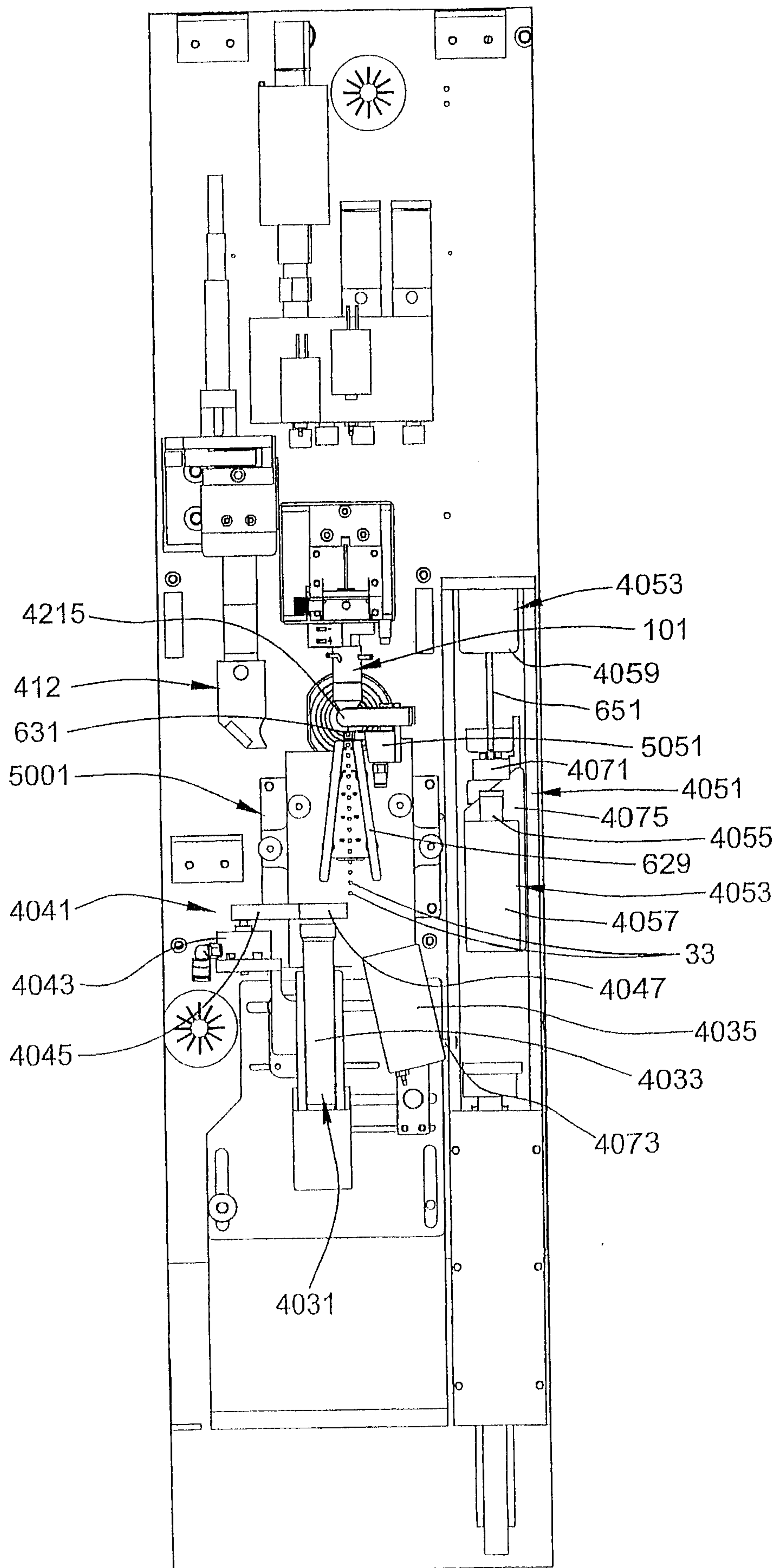


FIG. 25

FIG. 26

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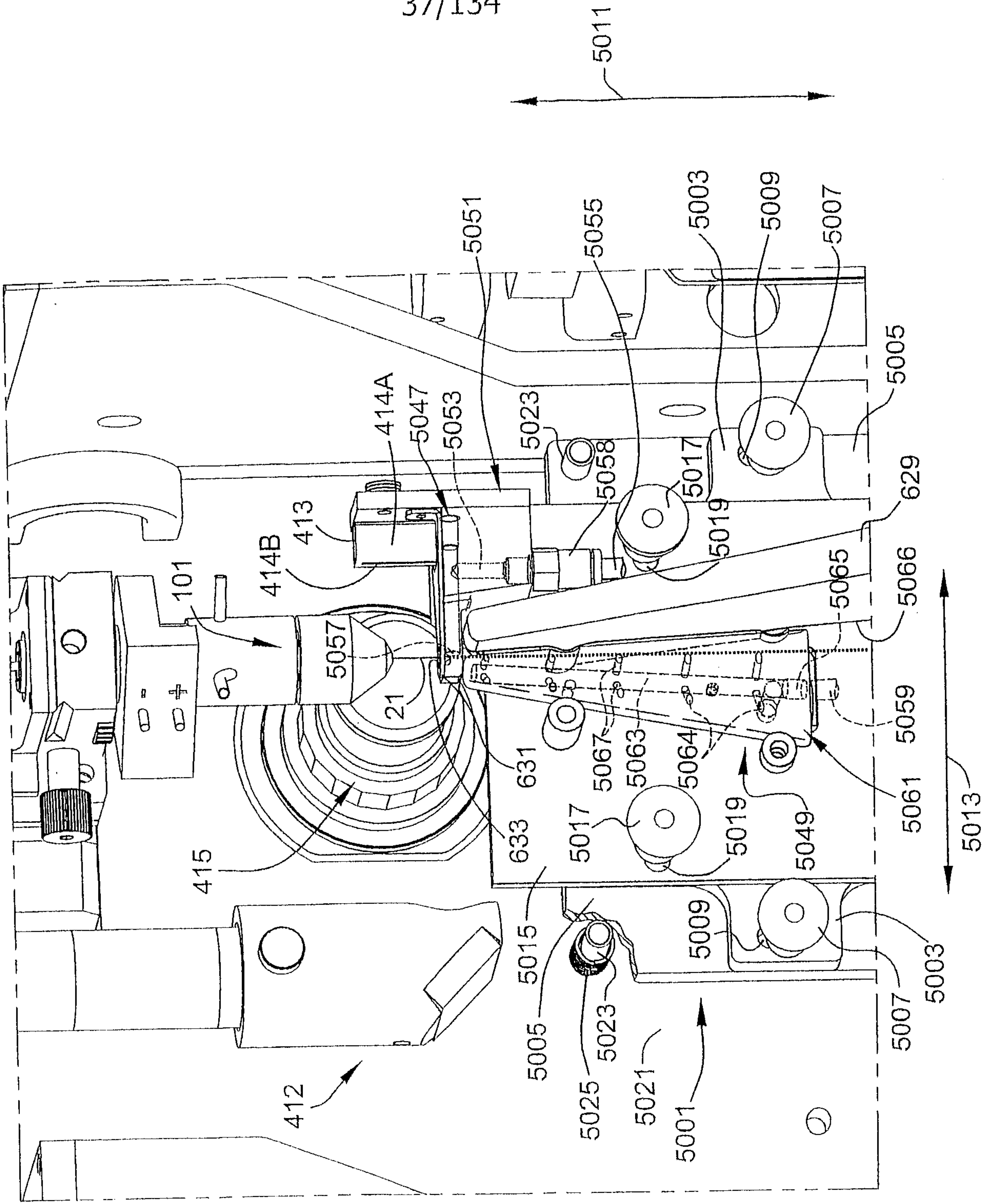


FIG. 27

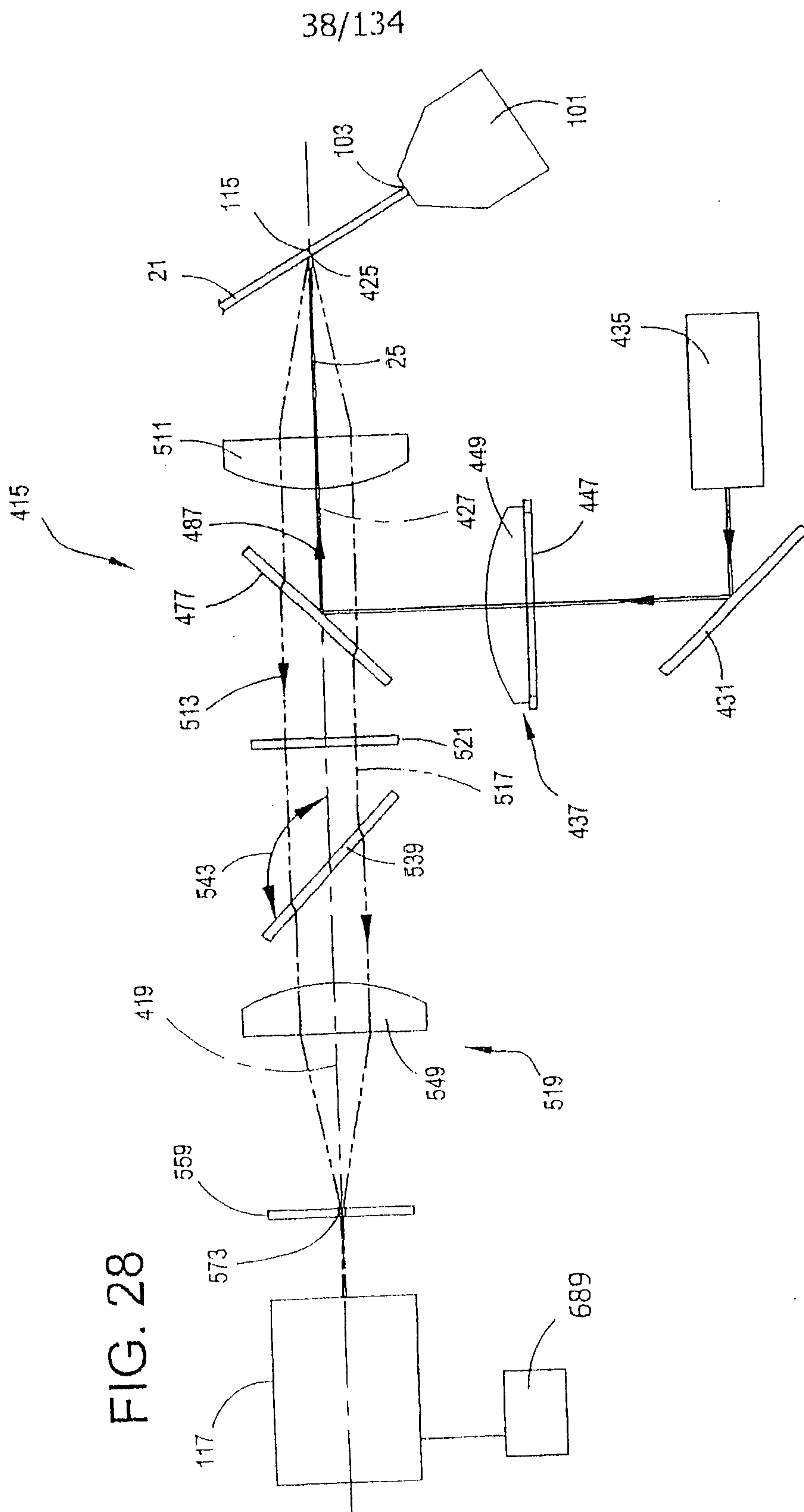
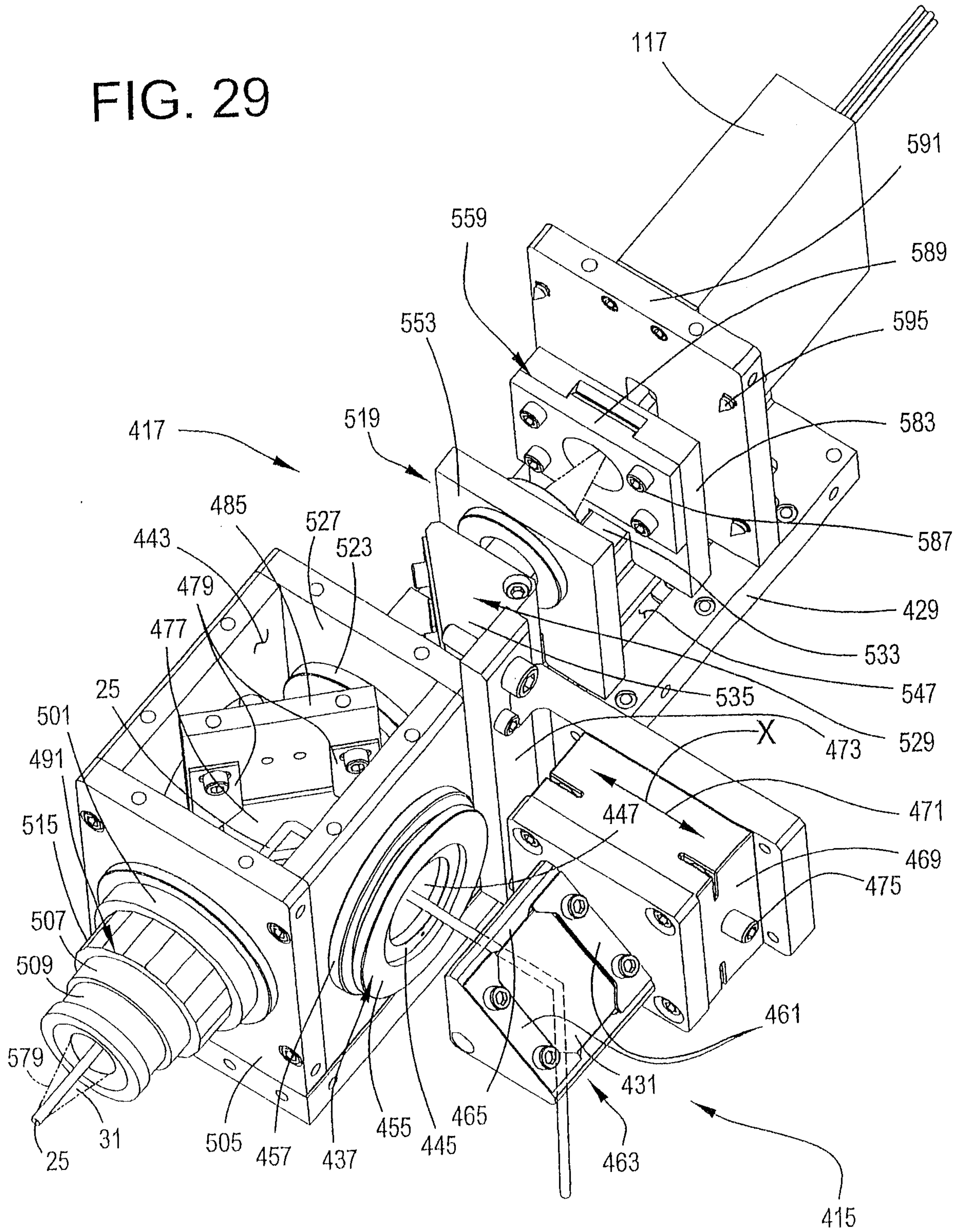
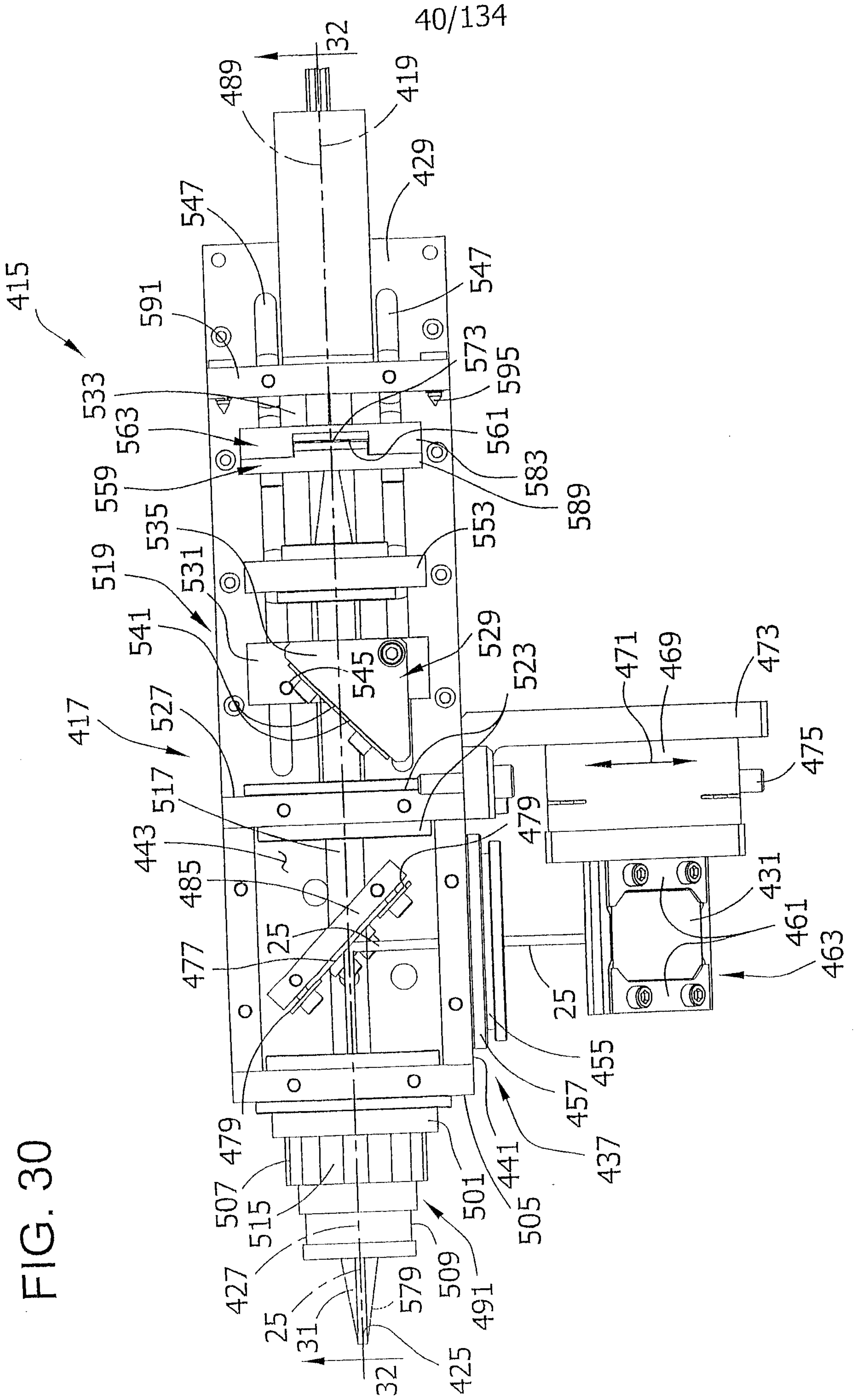


FIG. 29





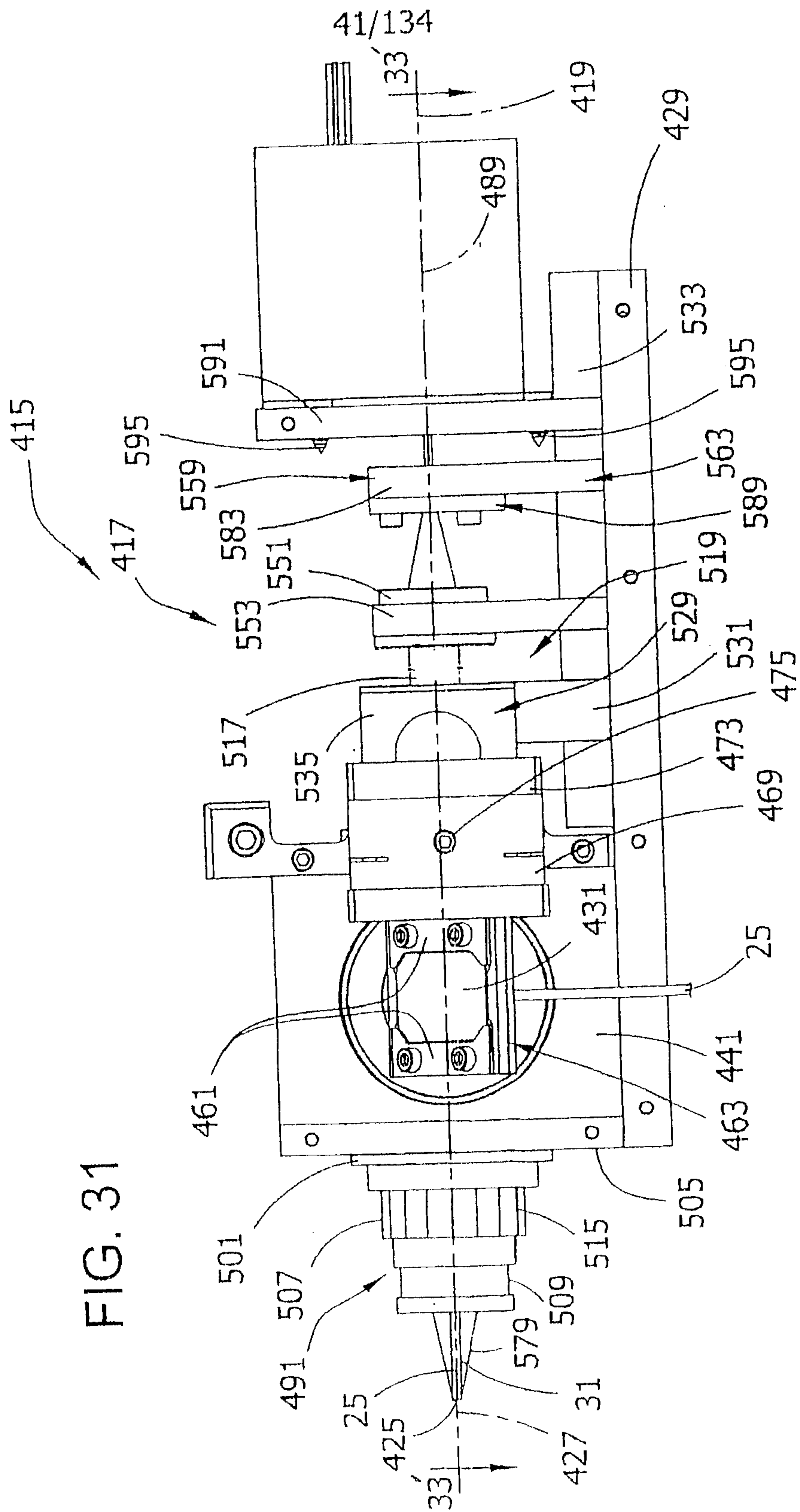


FIG. 31

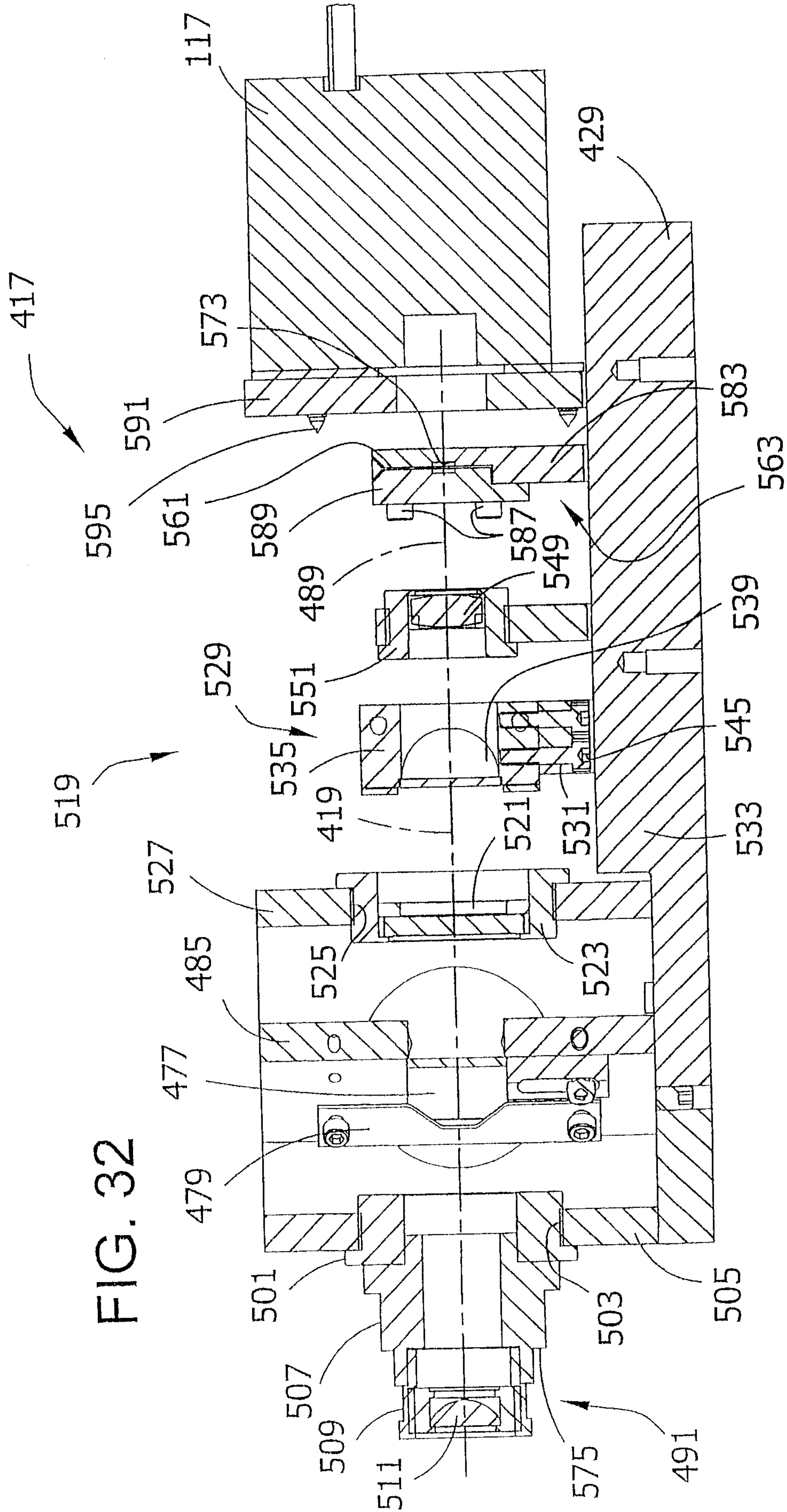
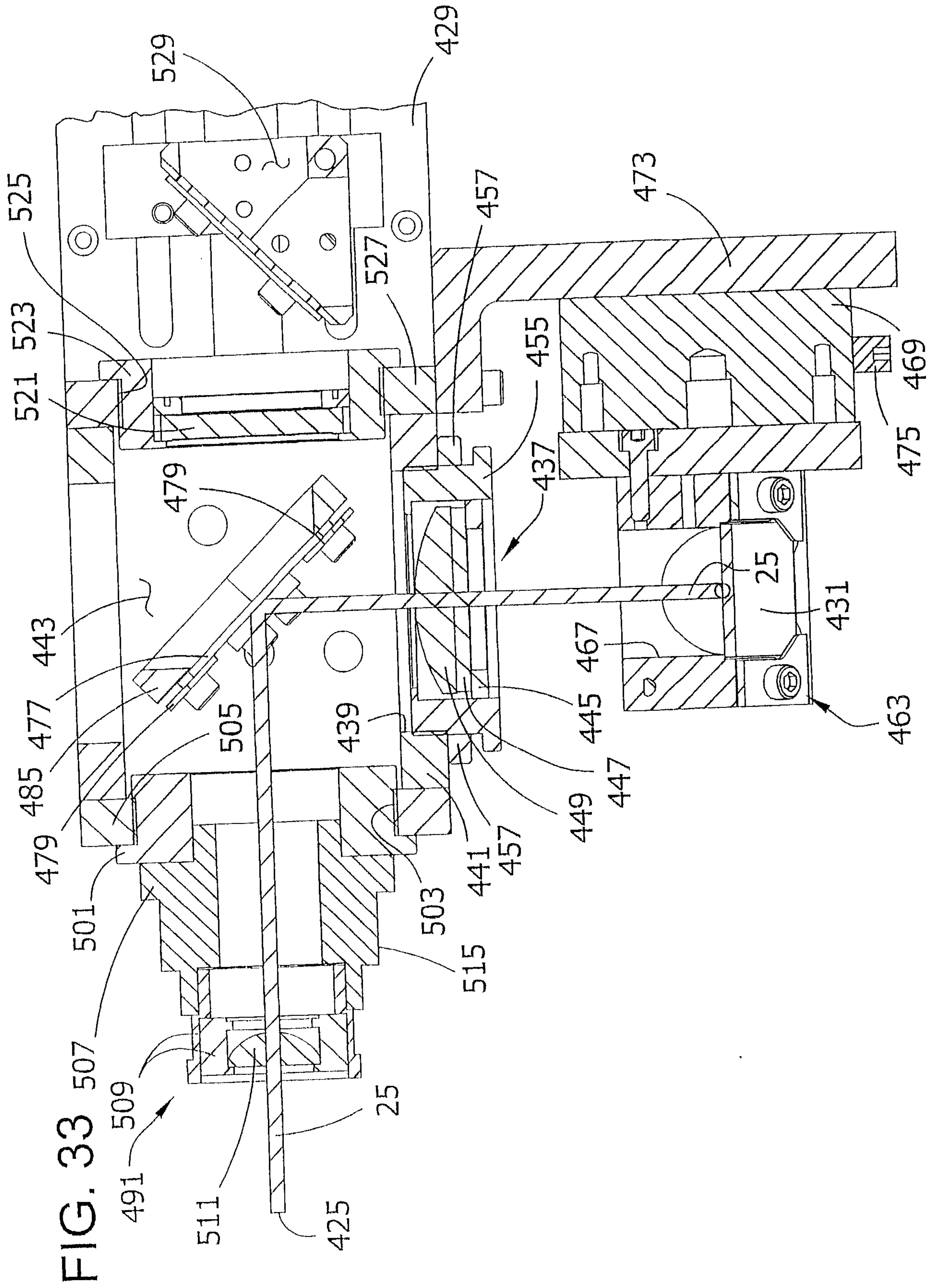
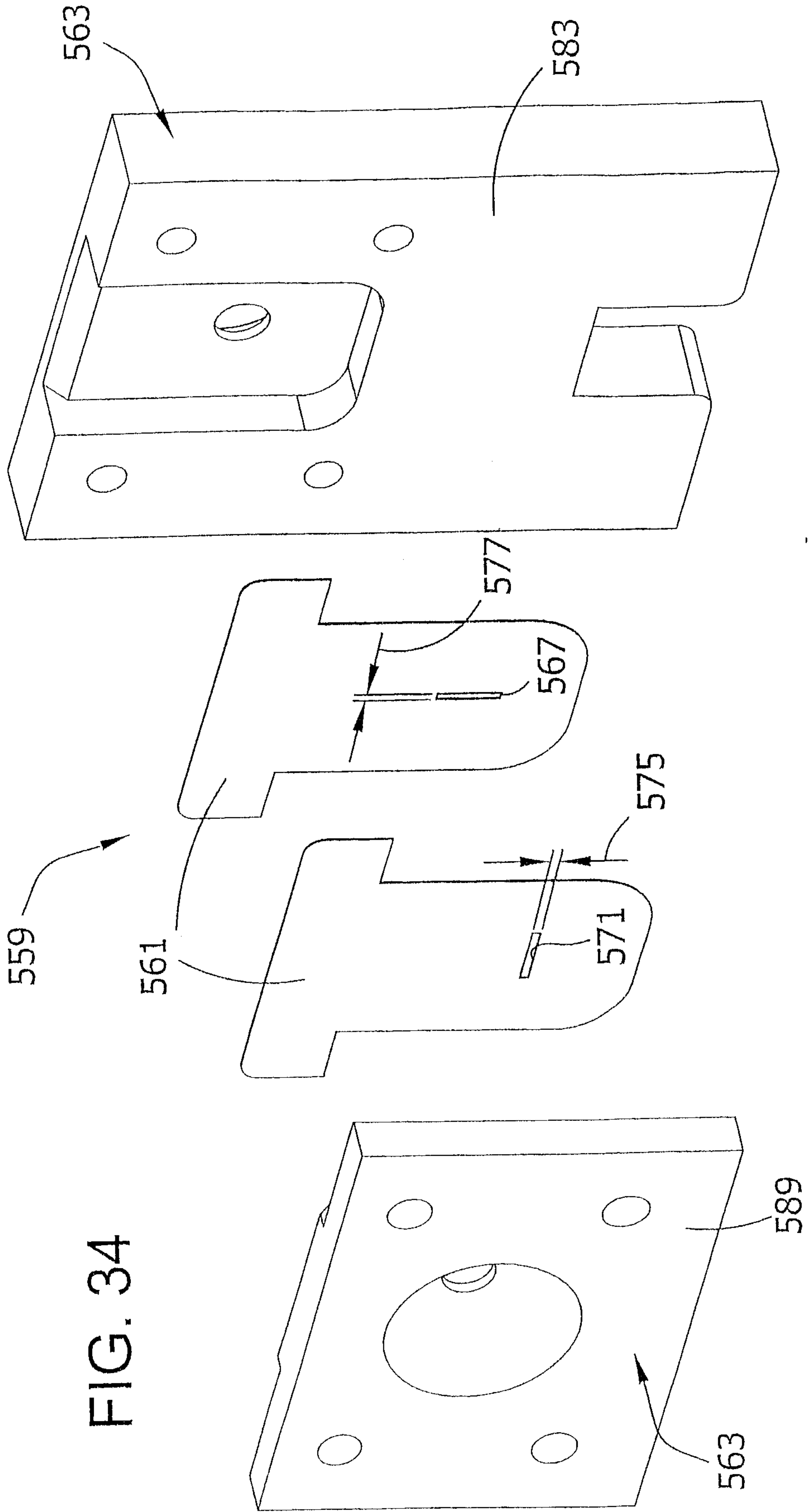


FIG. 32

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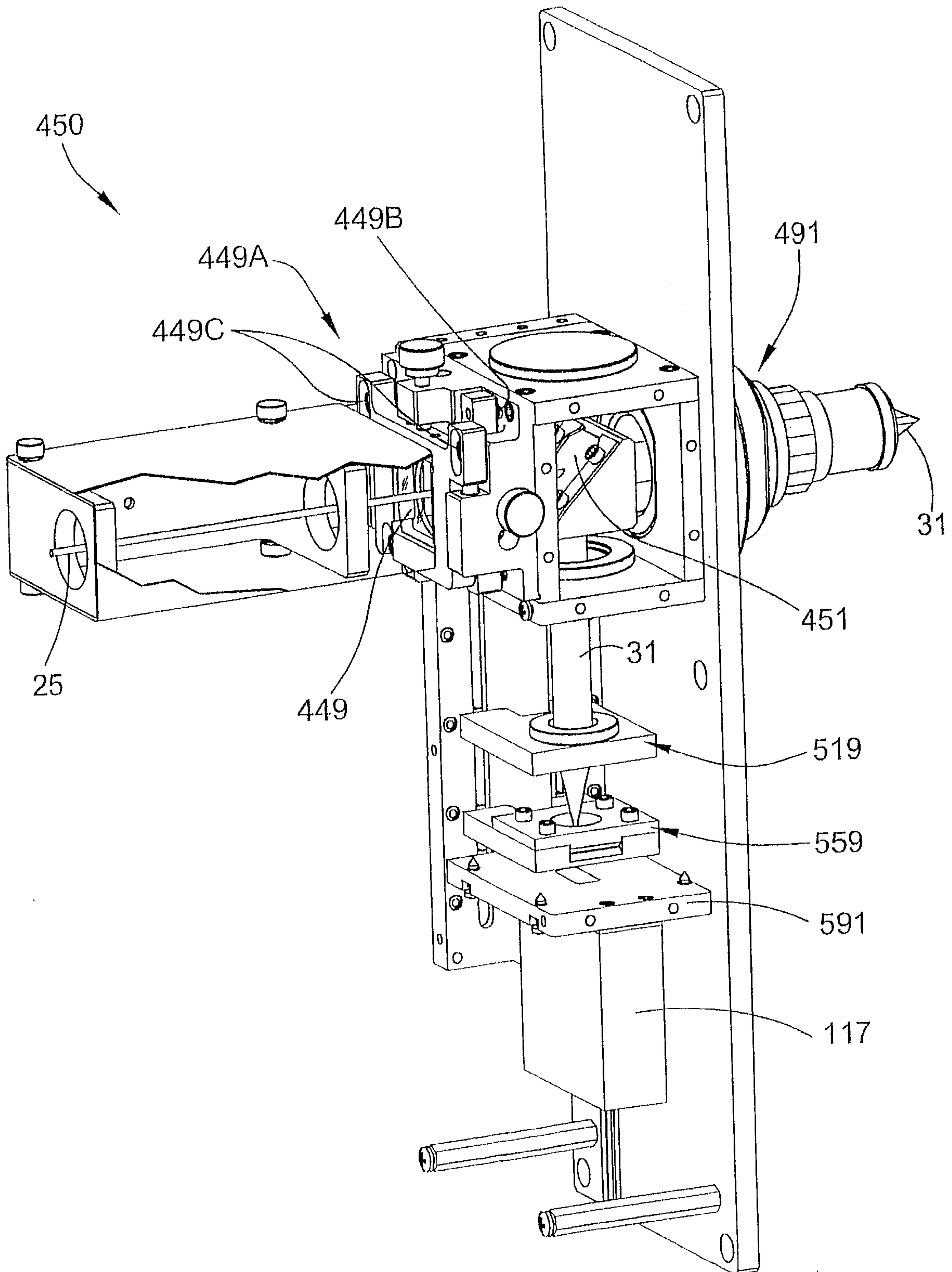


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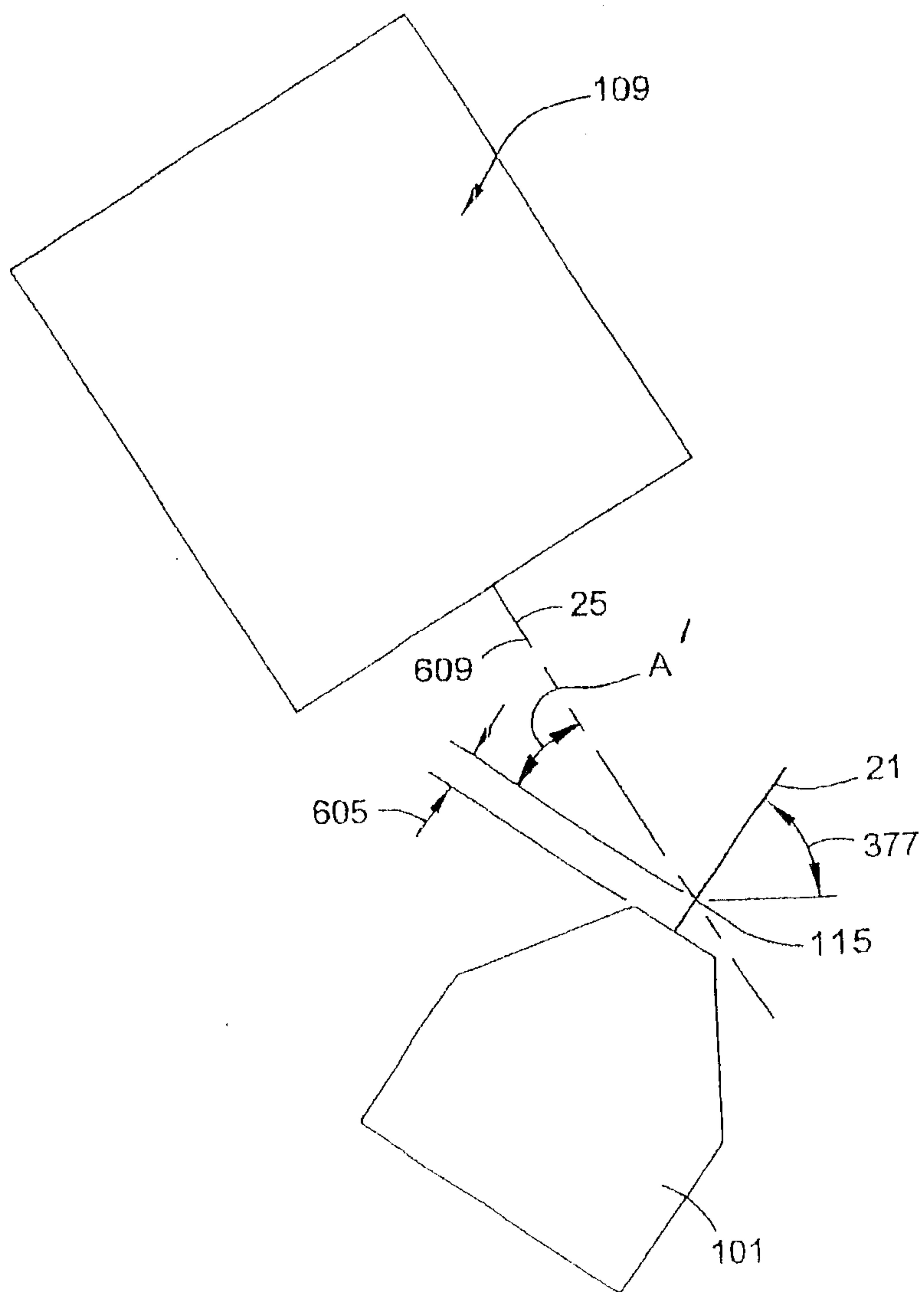
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FIG. 35



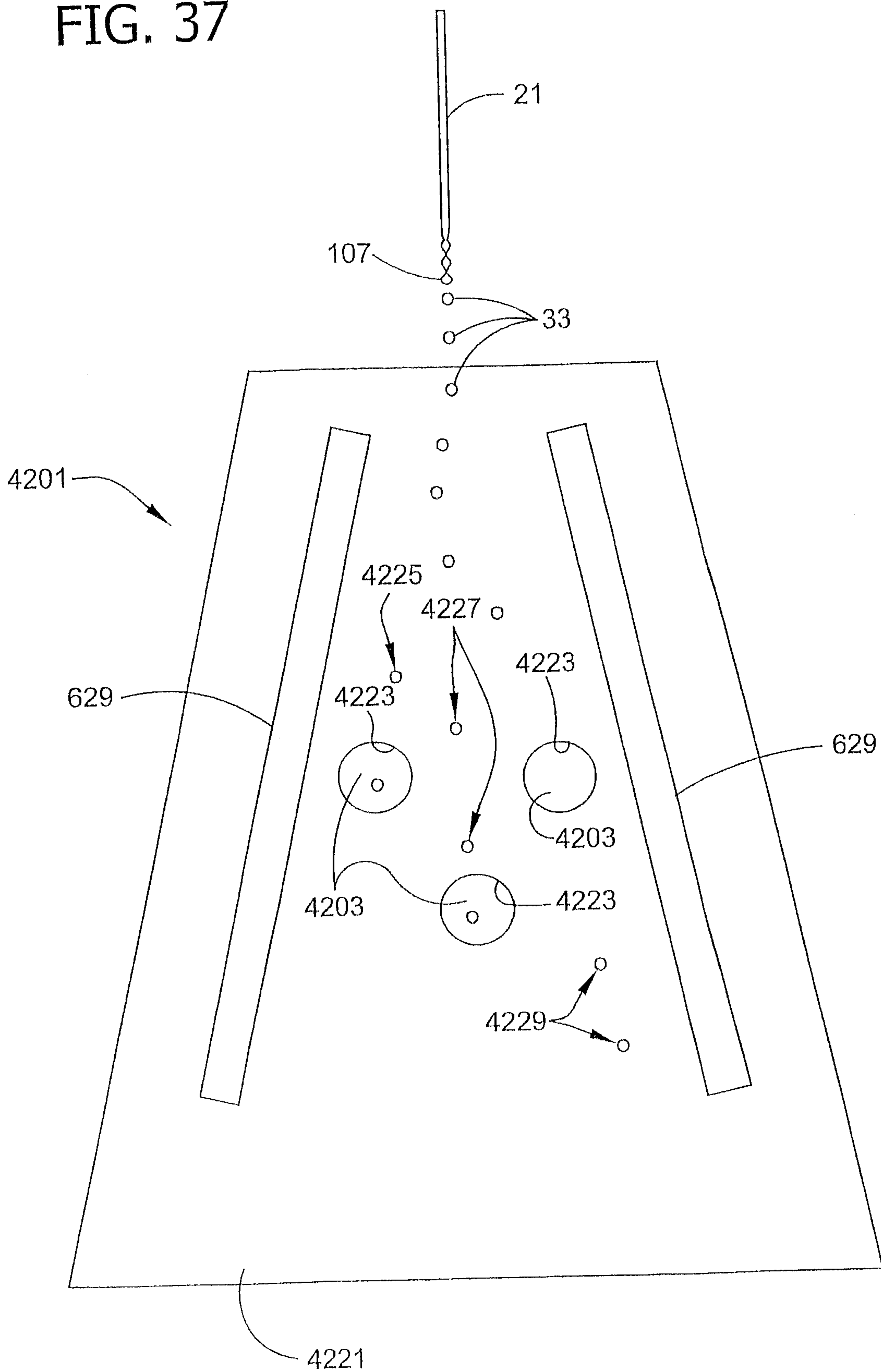
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FIG. 36



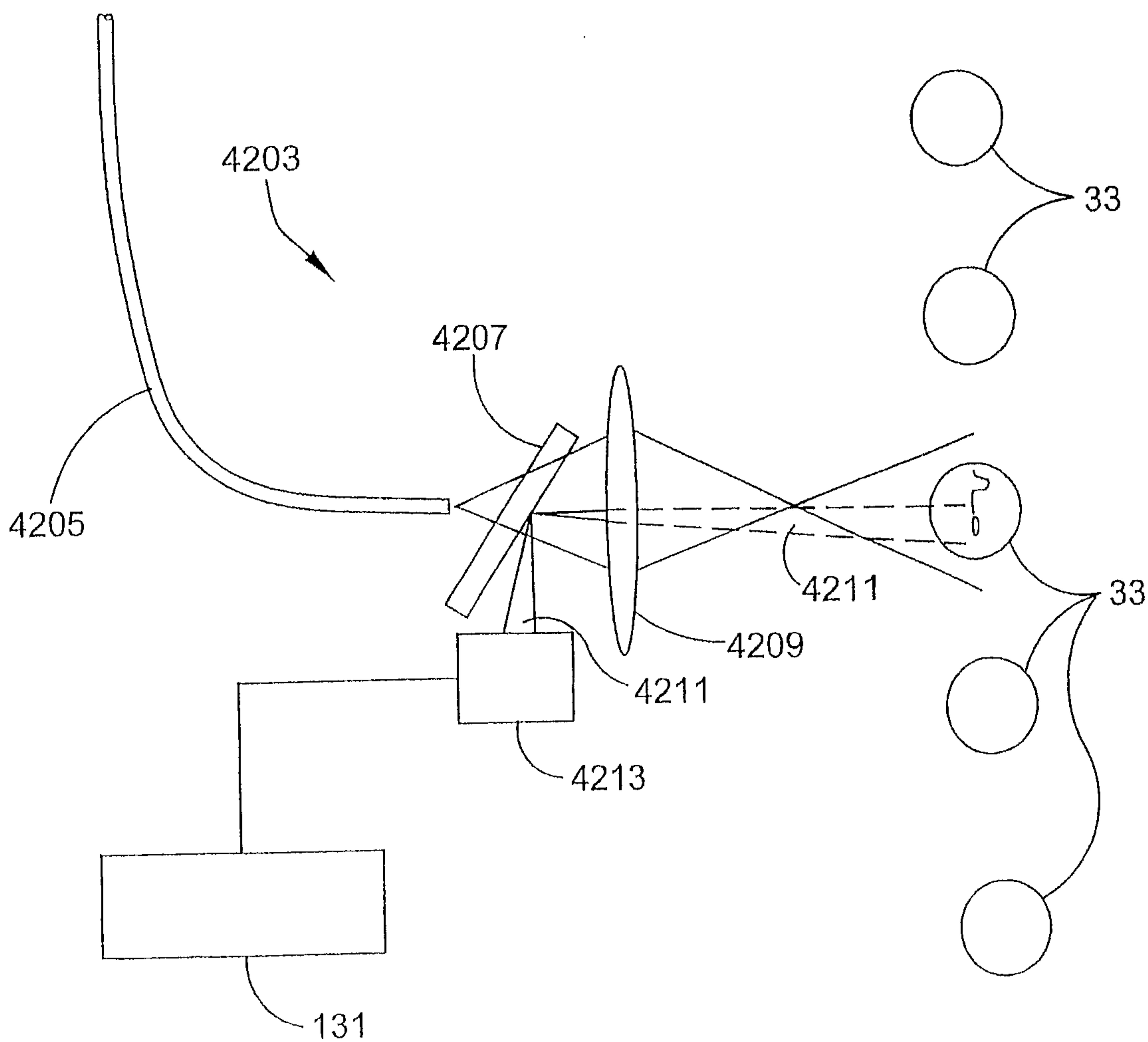
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FIG. 37



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FIG. 38



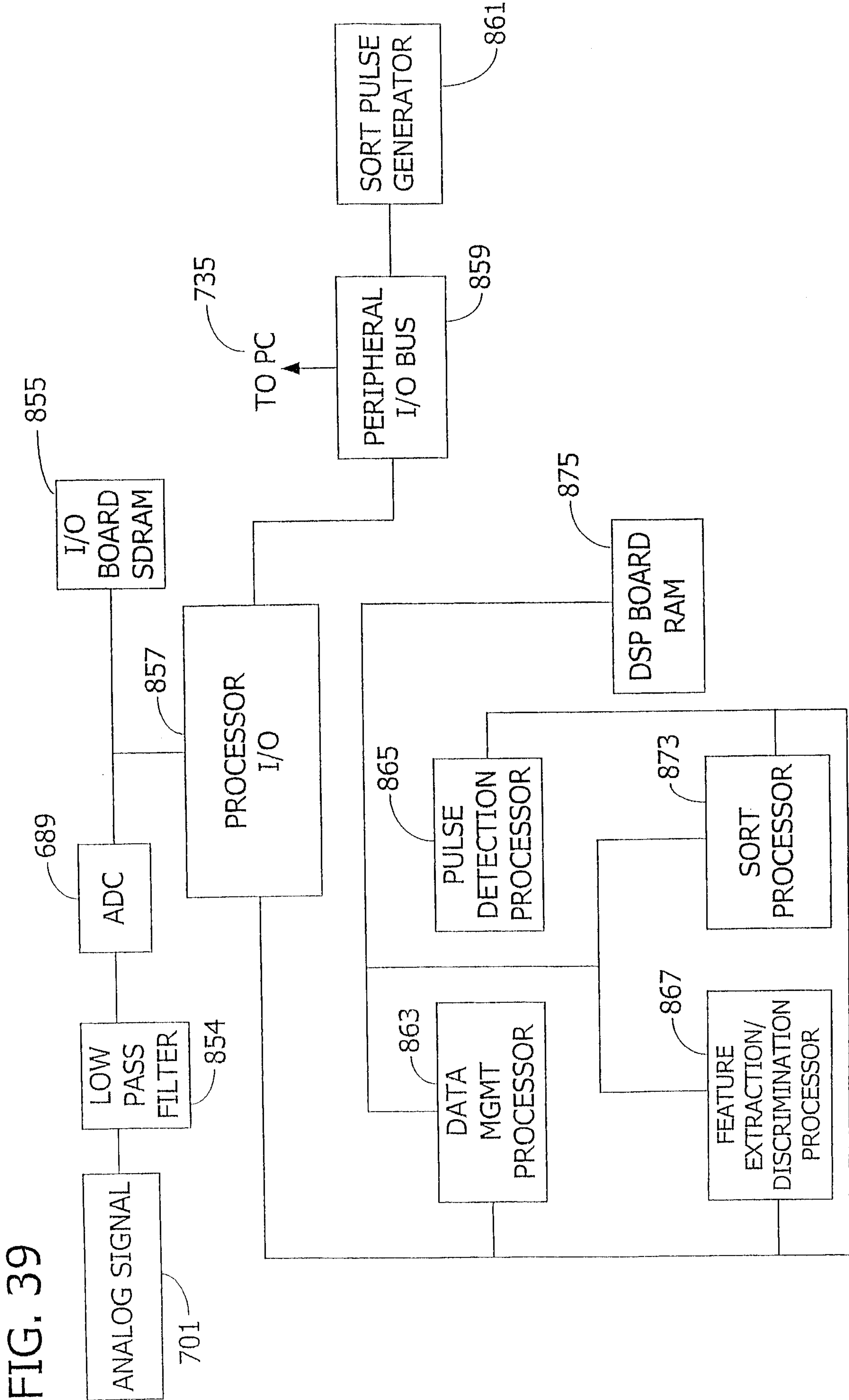


FIG. 39

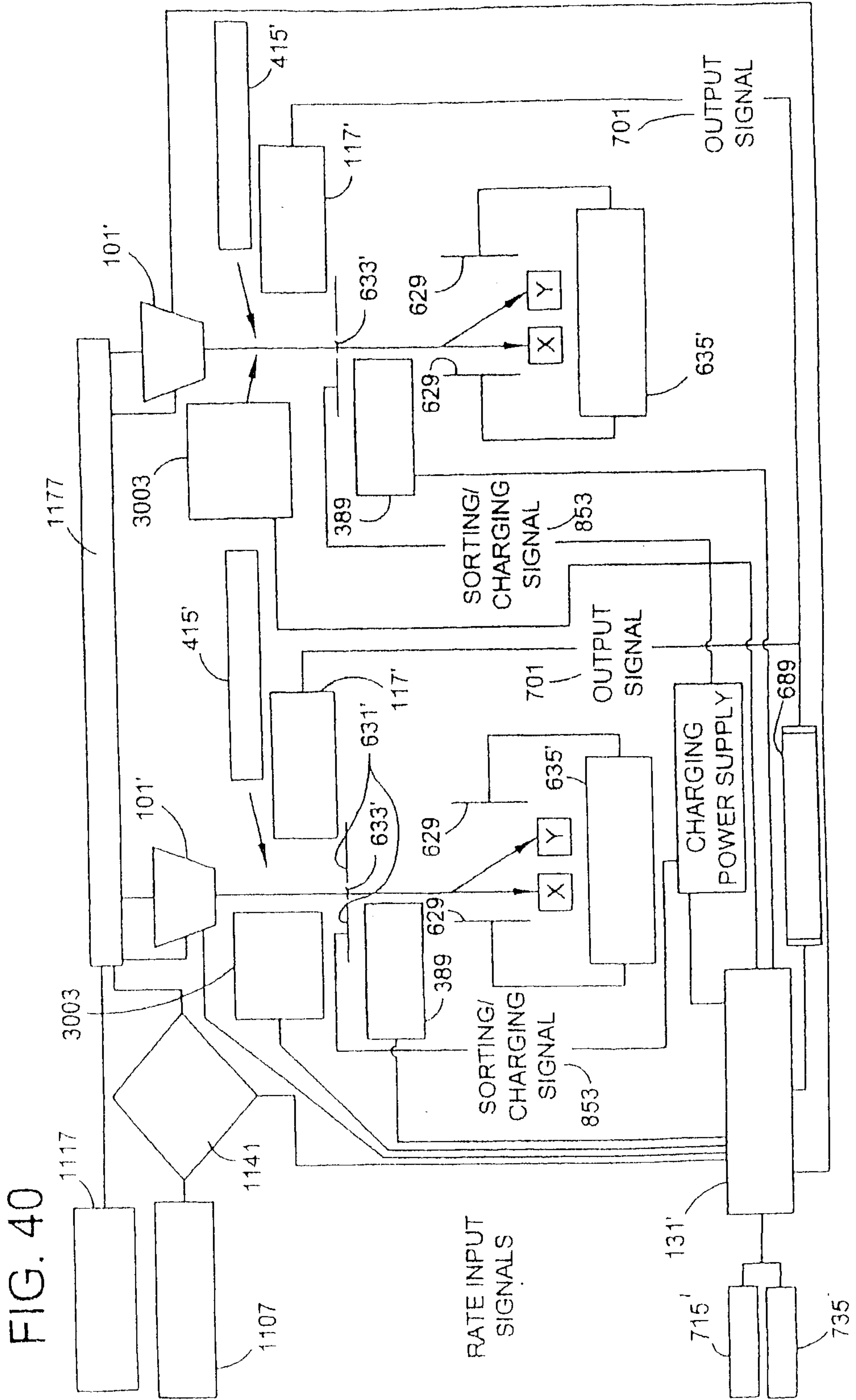


FIG. 40

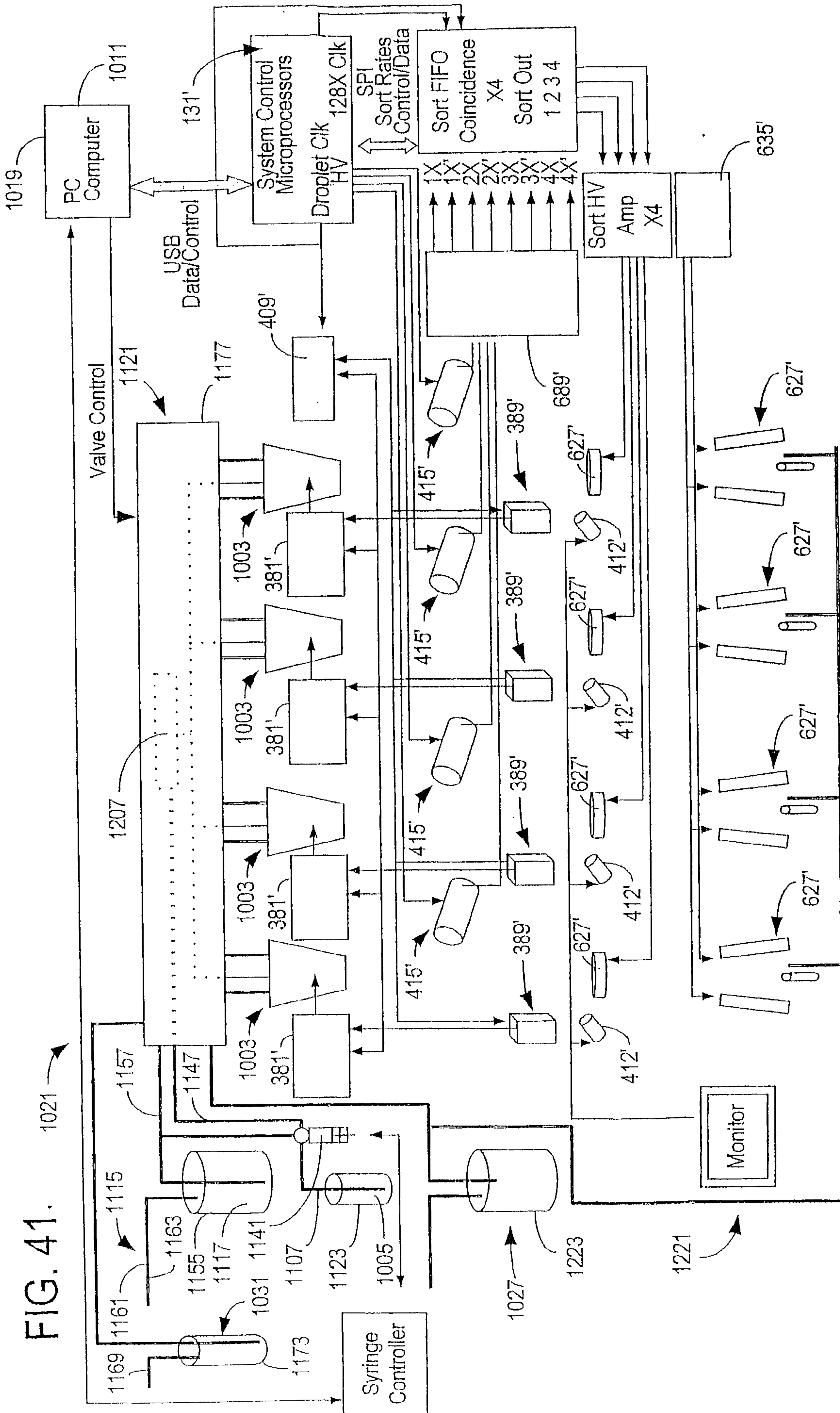
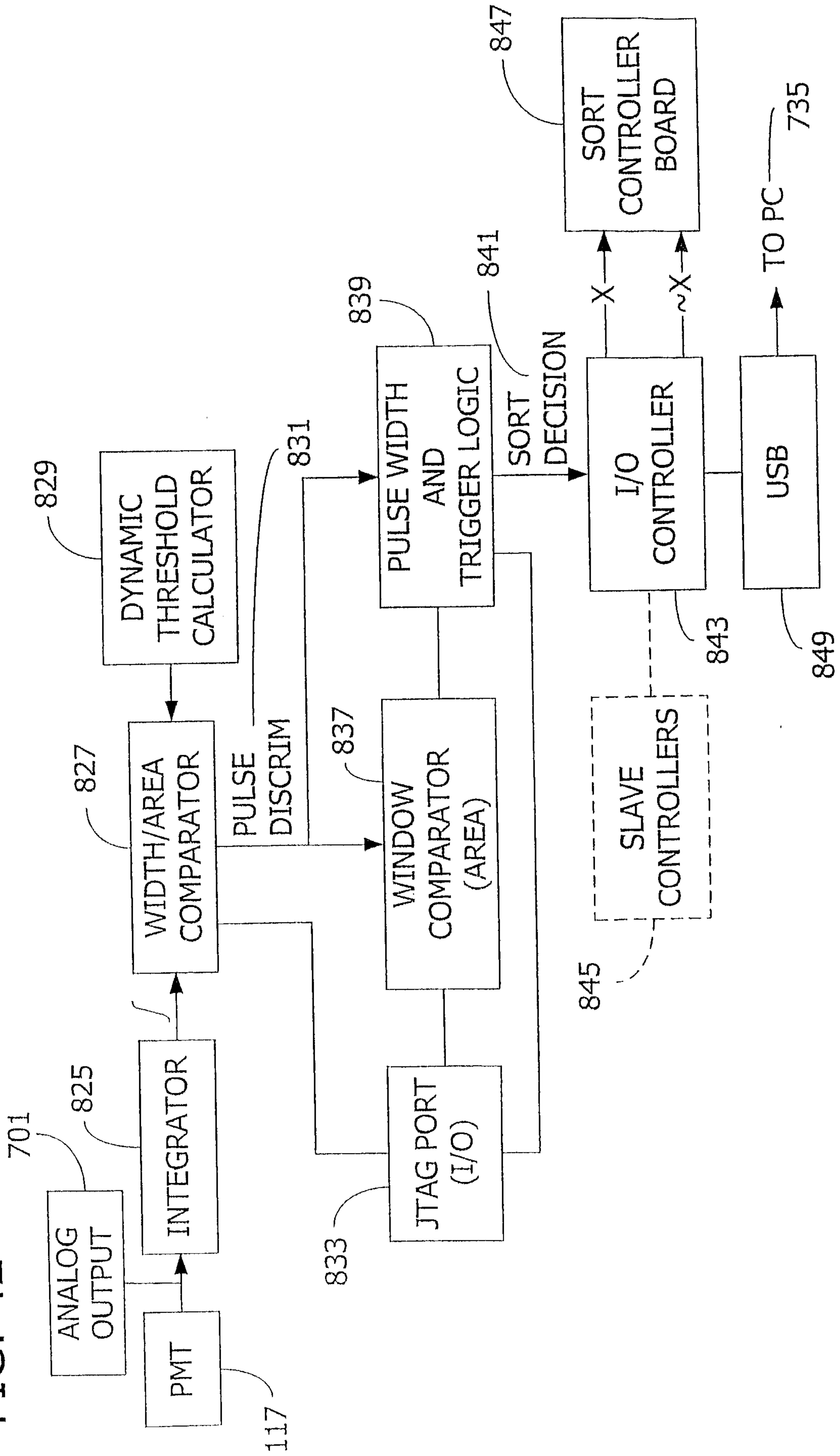


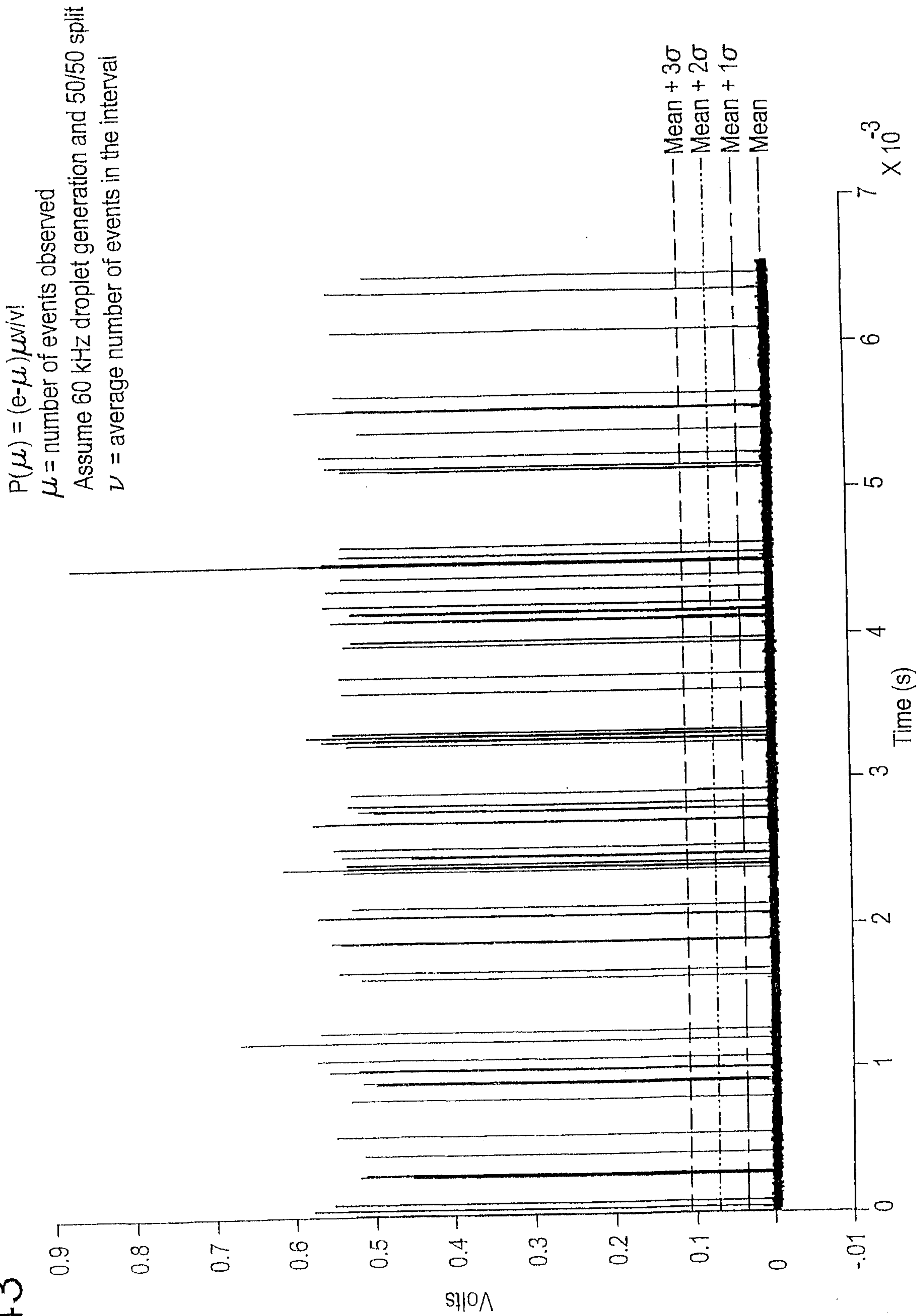
FIG. 41.

FIG. 42

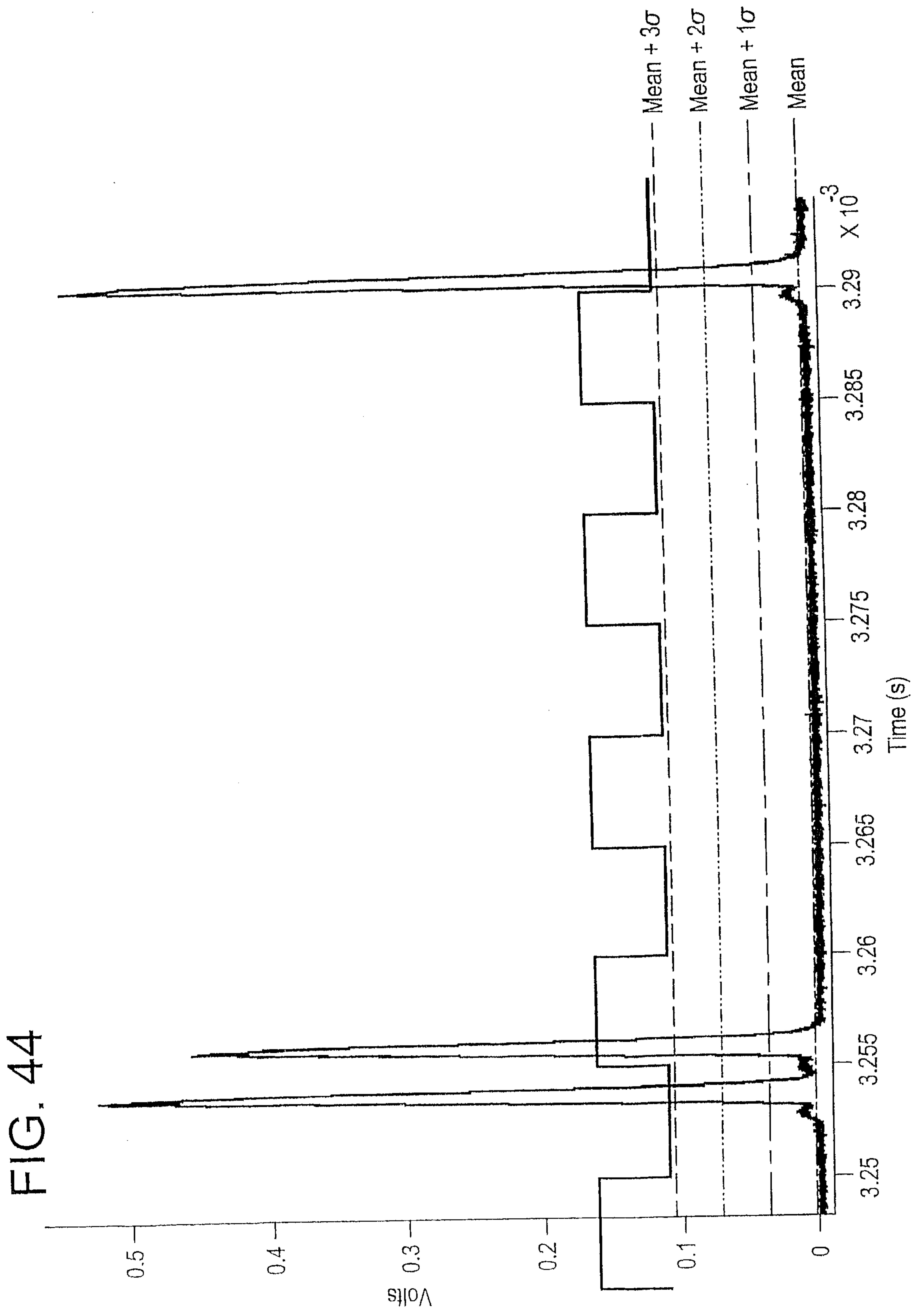


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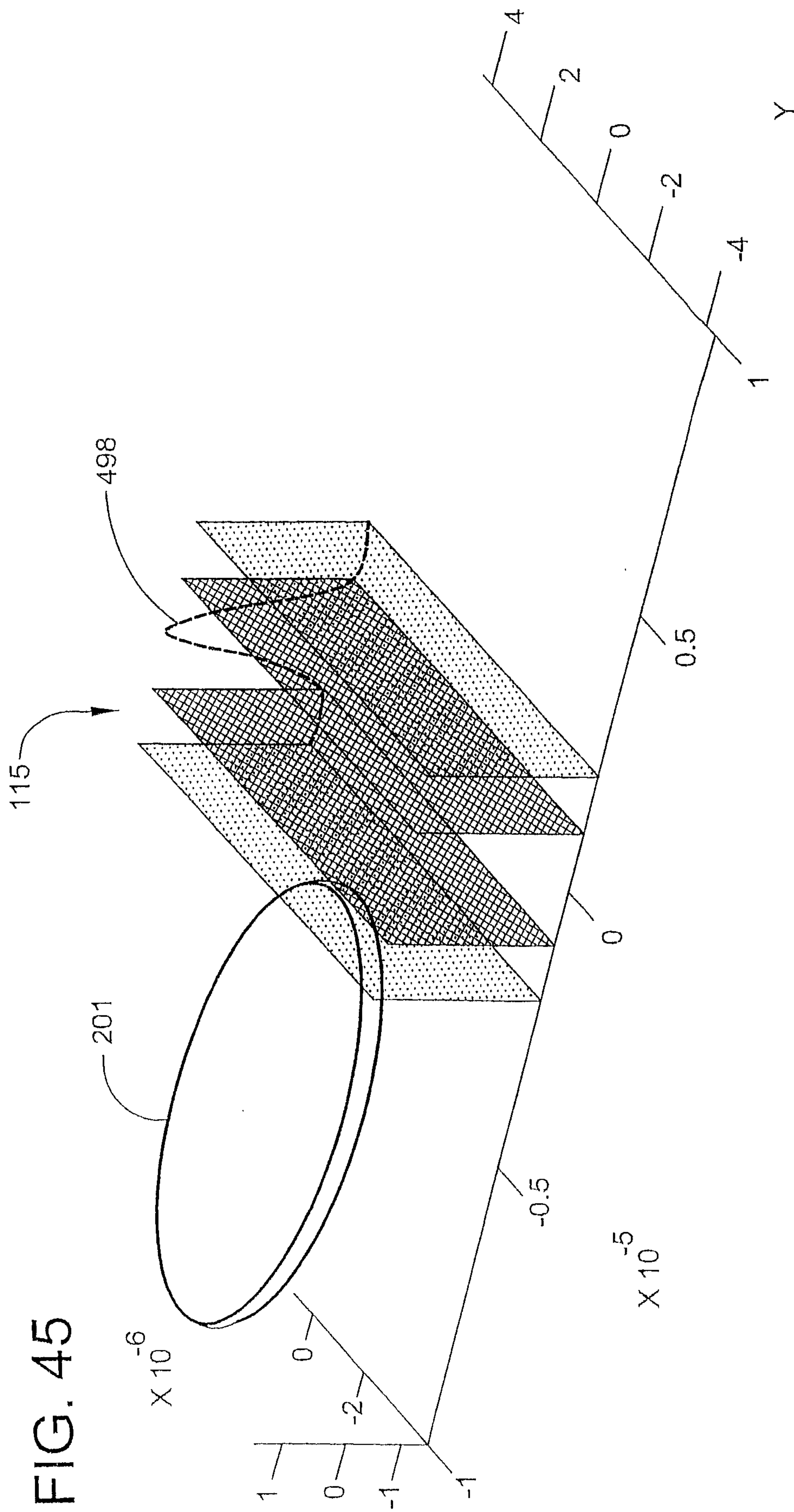
FIG. 43



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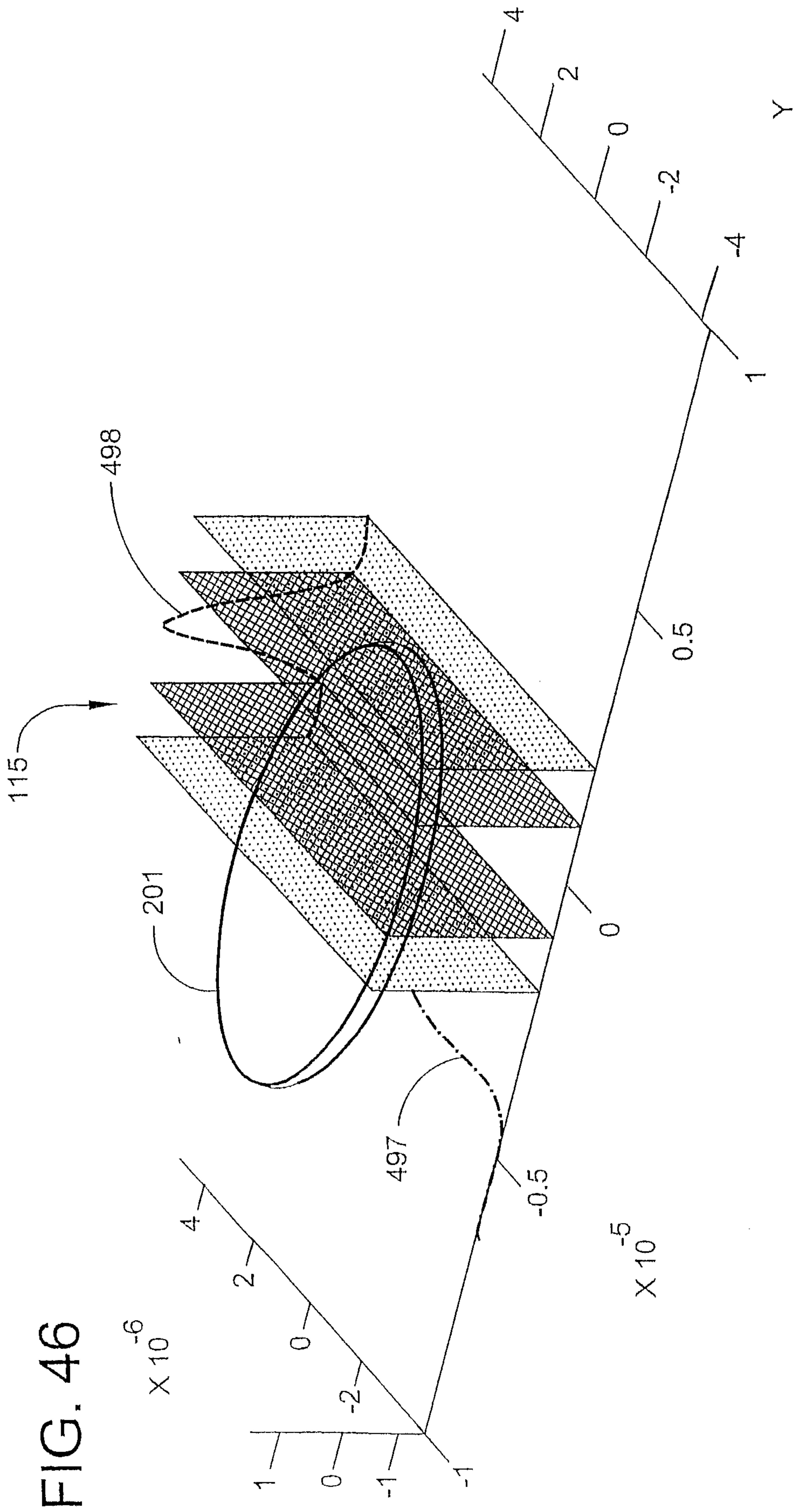
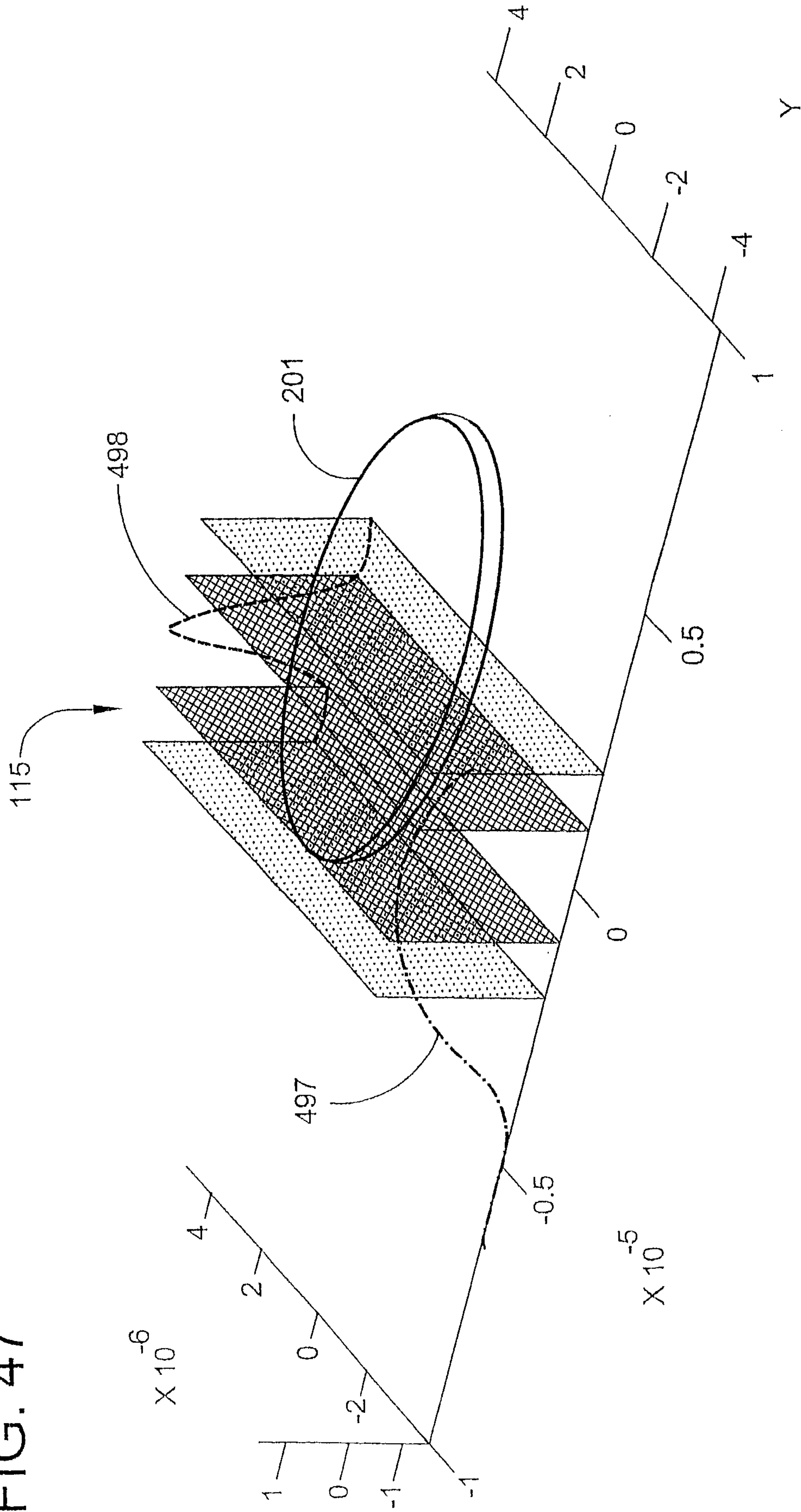


FIG. 46

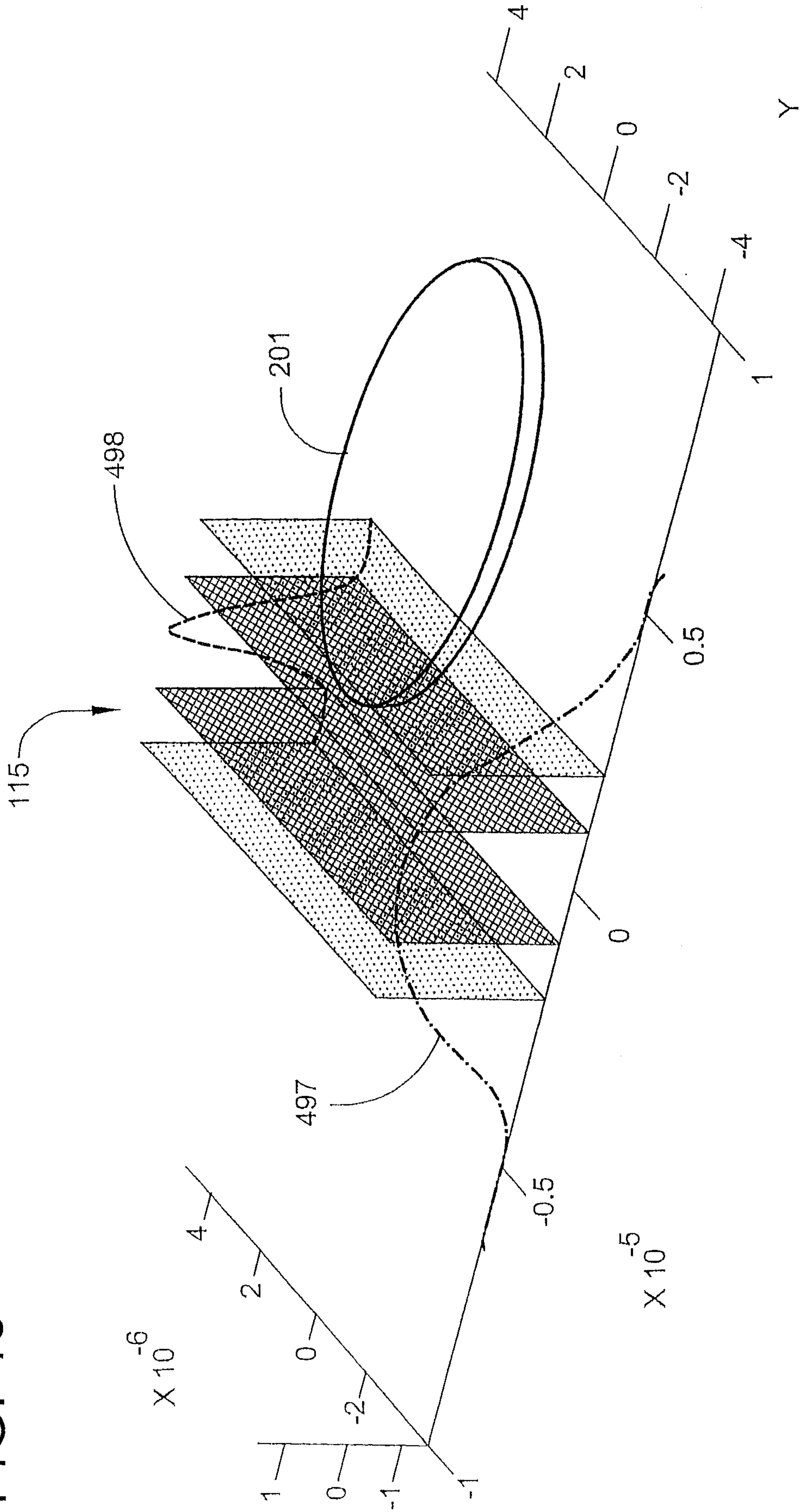
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FIG. 47

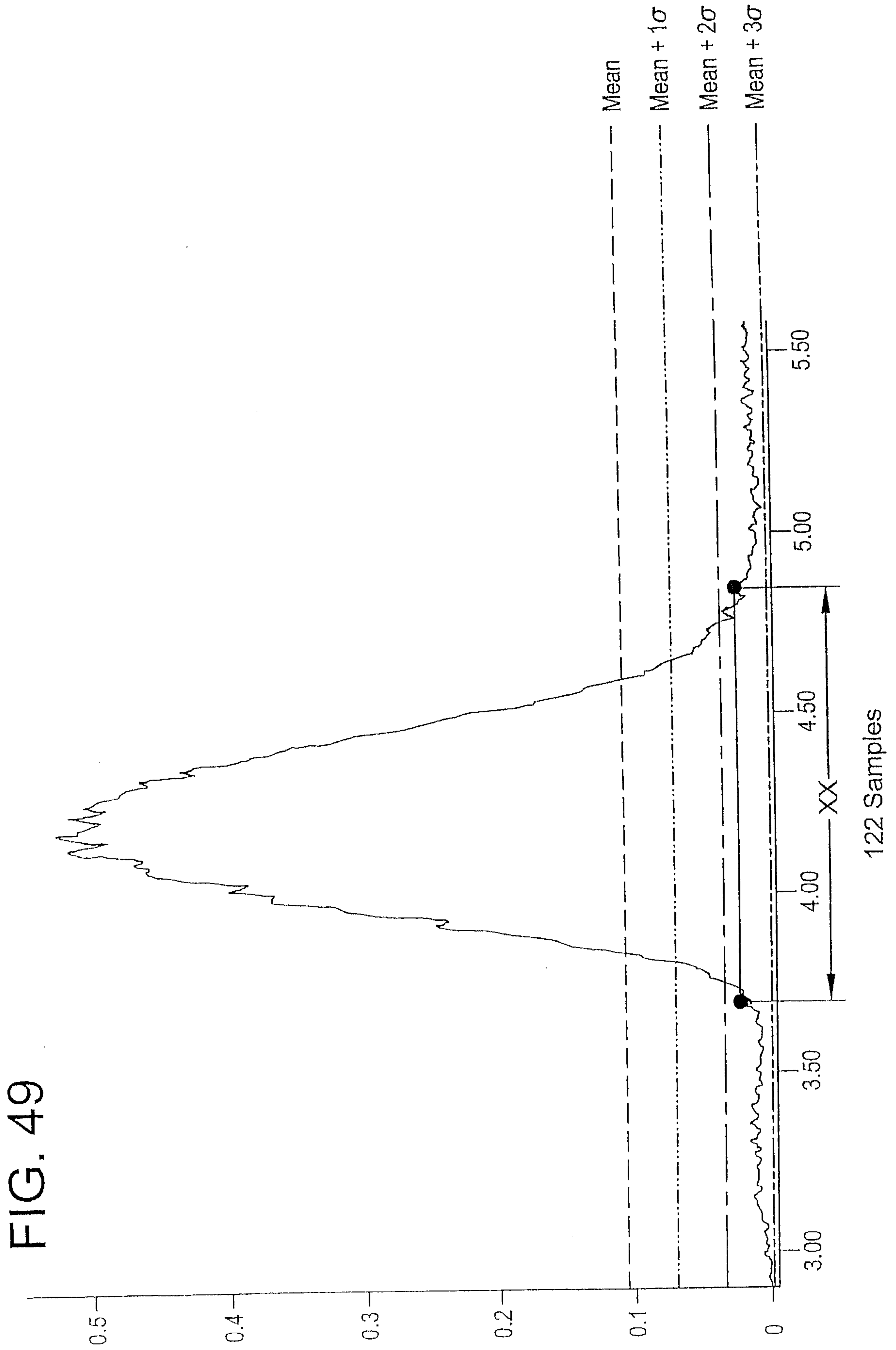


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FIG. 48



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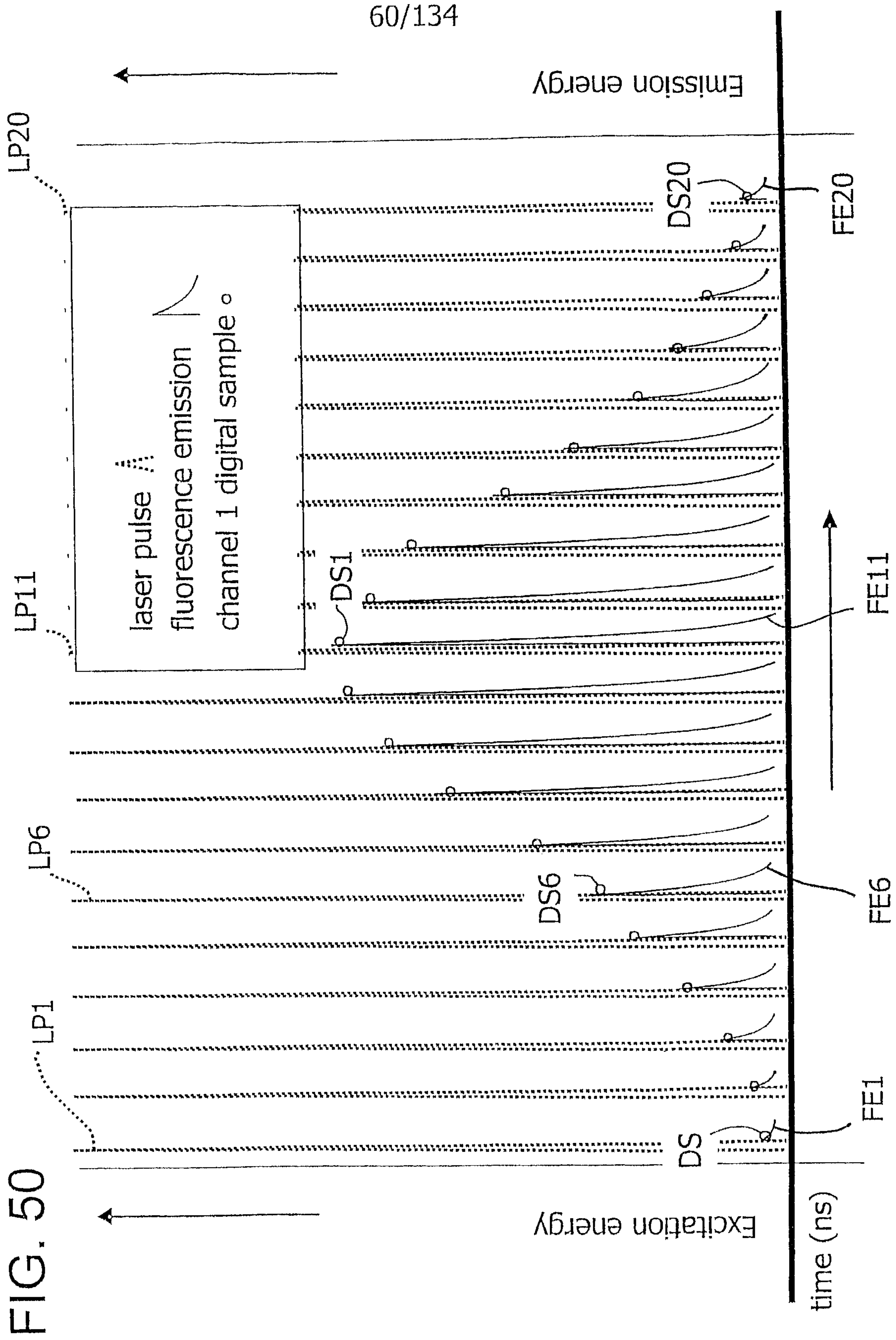
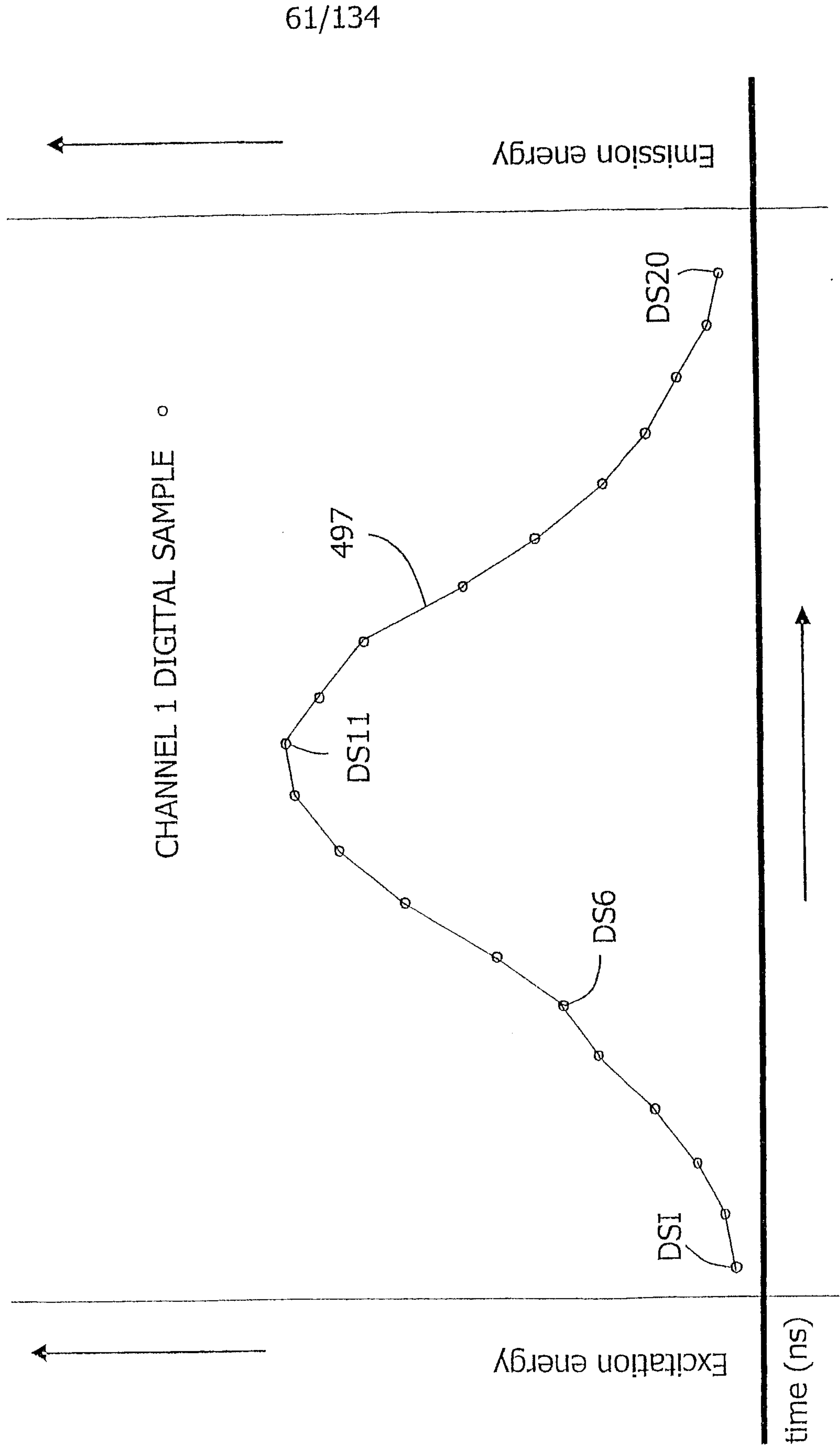


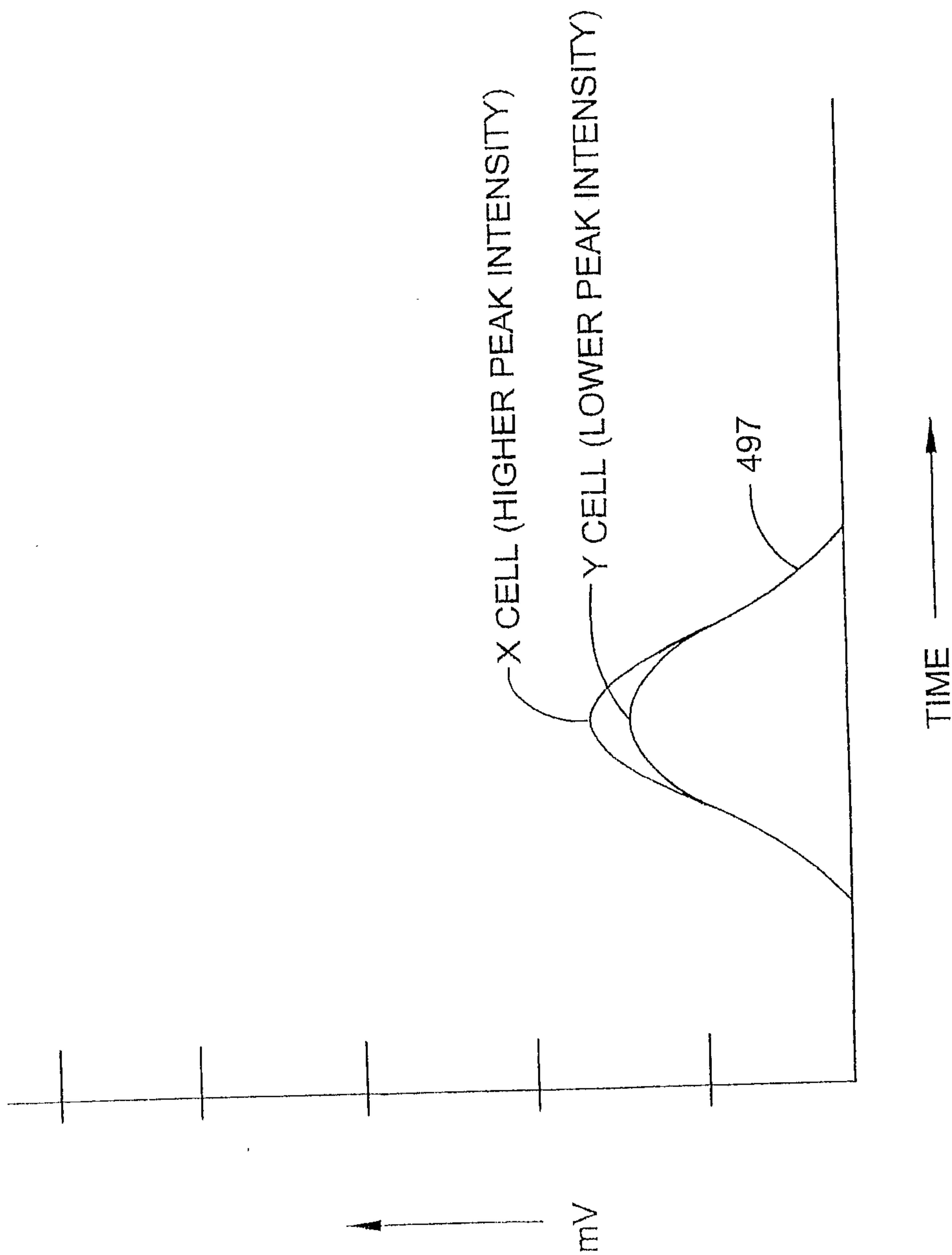
FIG. 50

FIG. 51



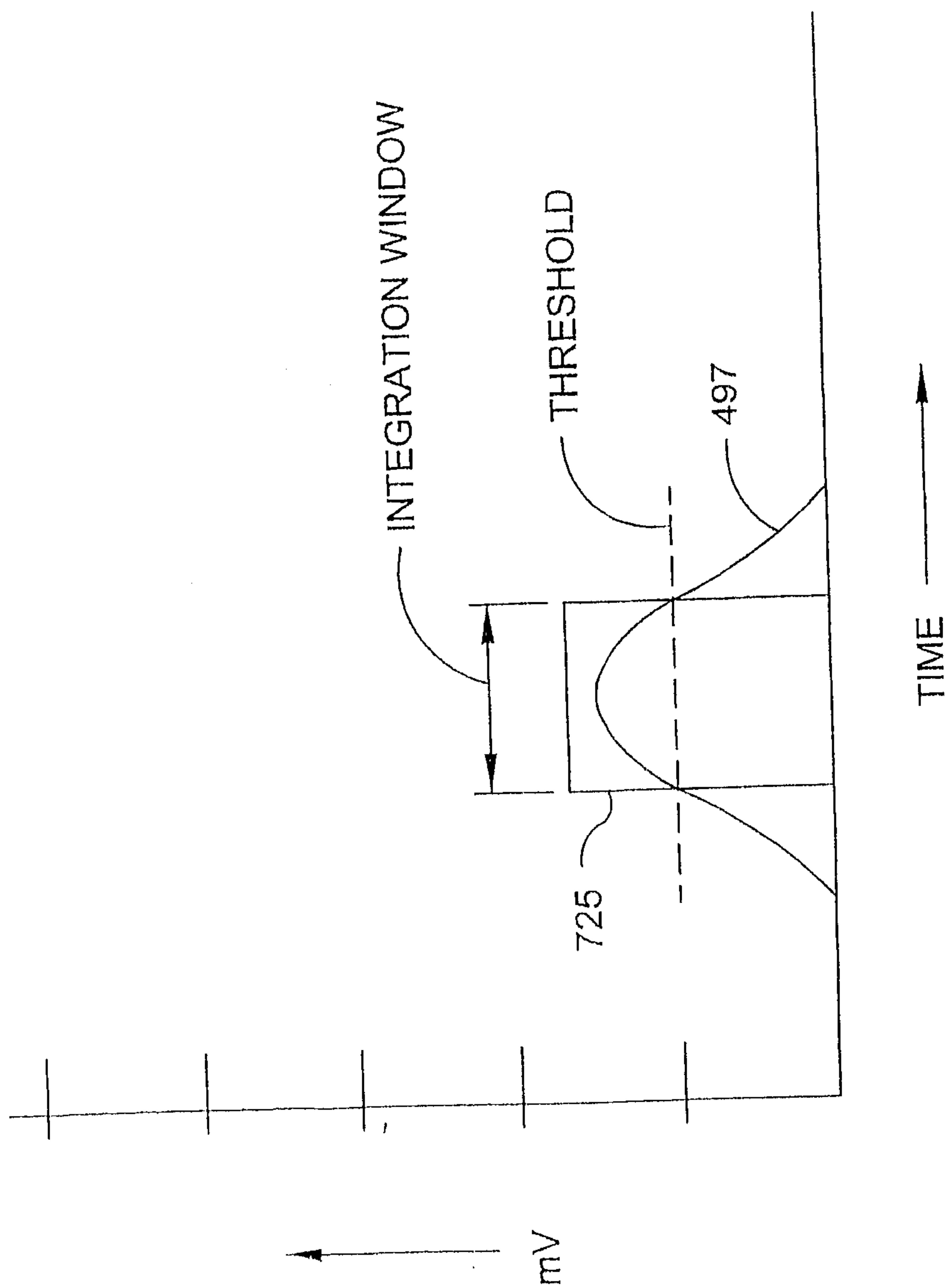
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FIG. 52



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FIG. 53



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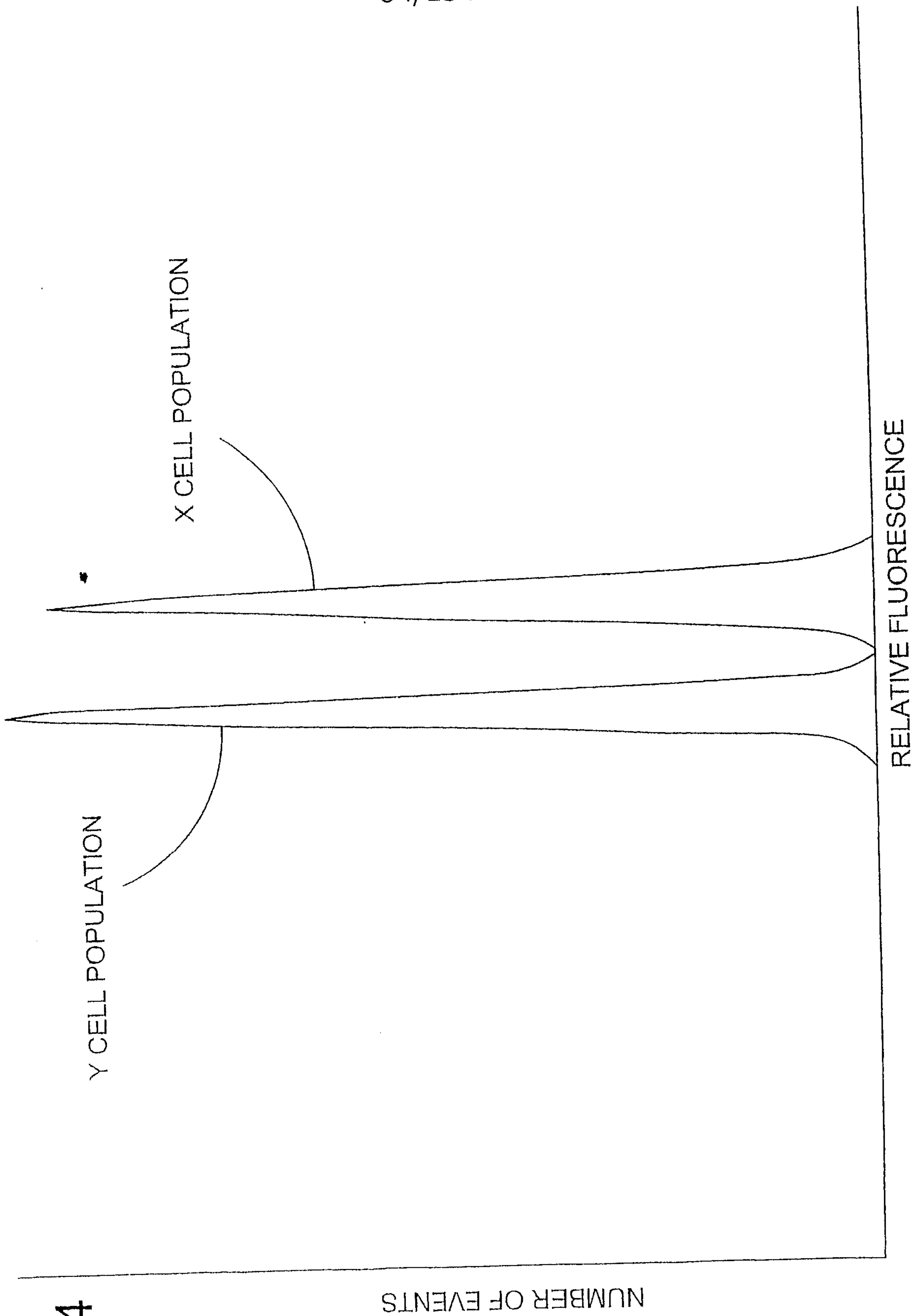


FIG. 54

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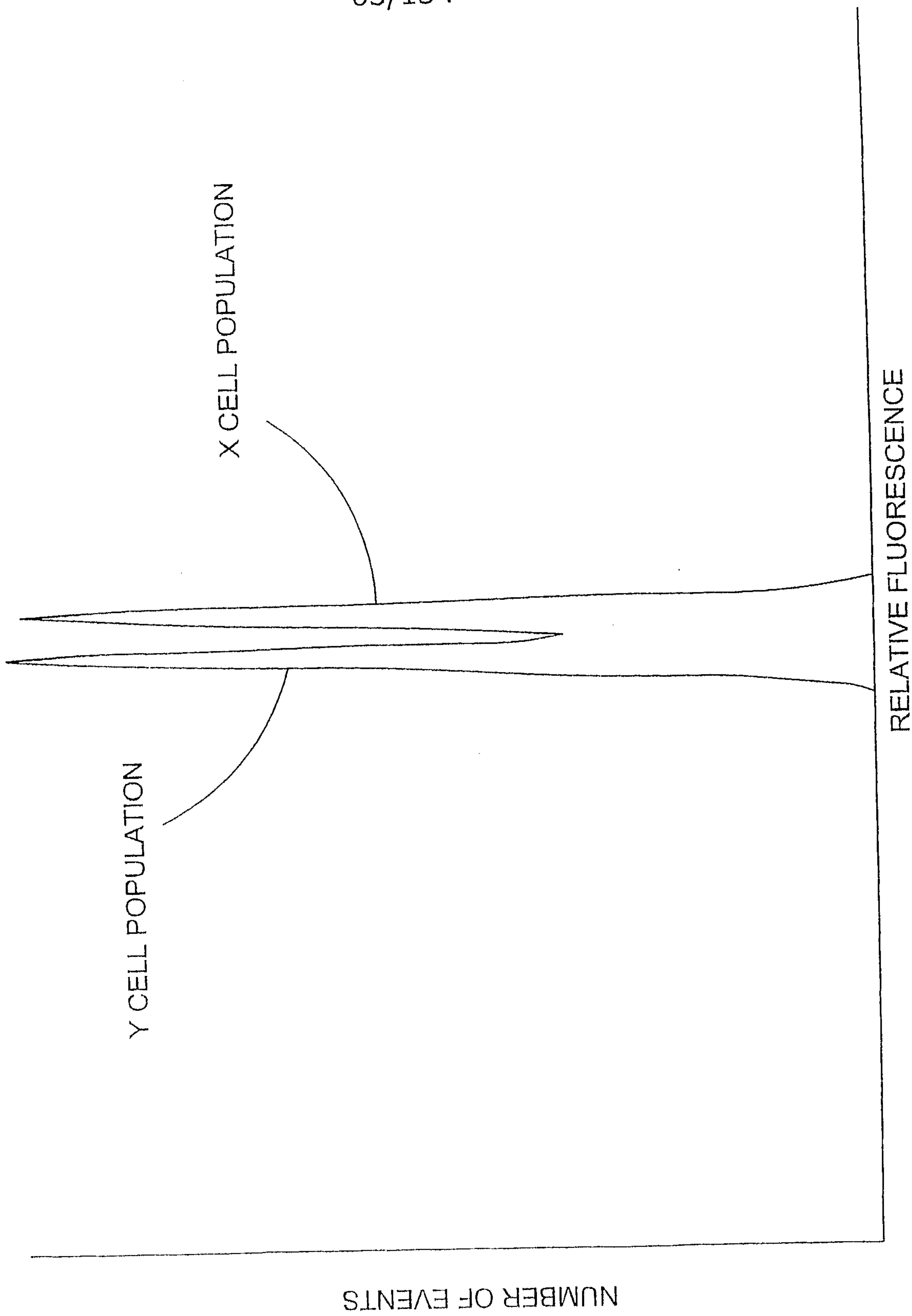


FIG. 55

FIG. 57

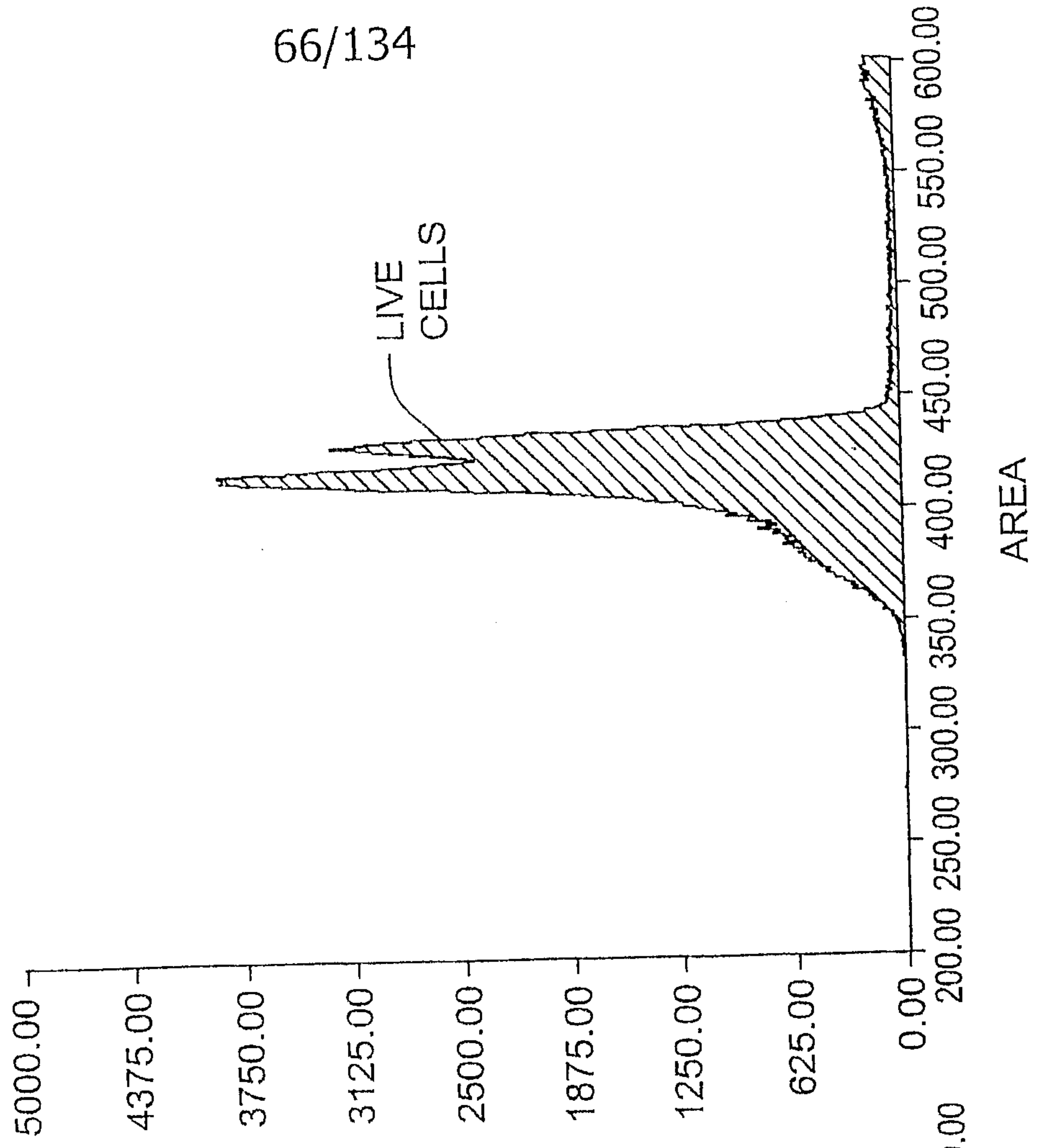


FIG. 56

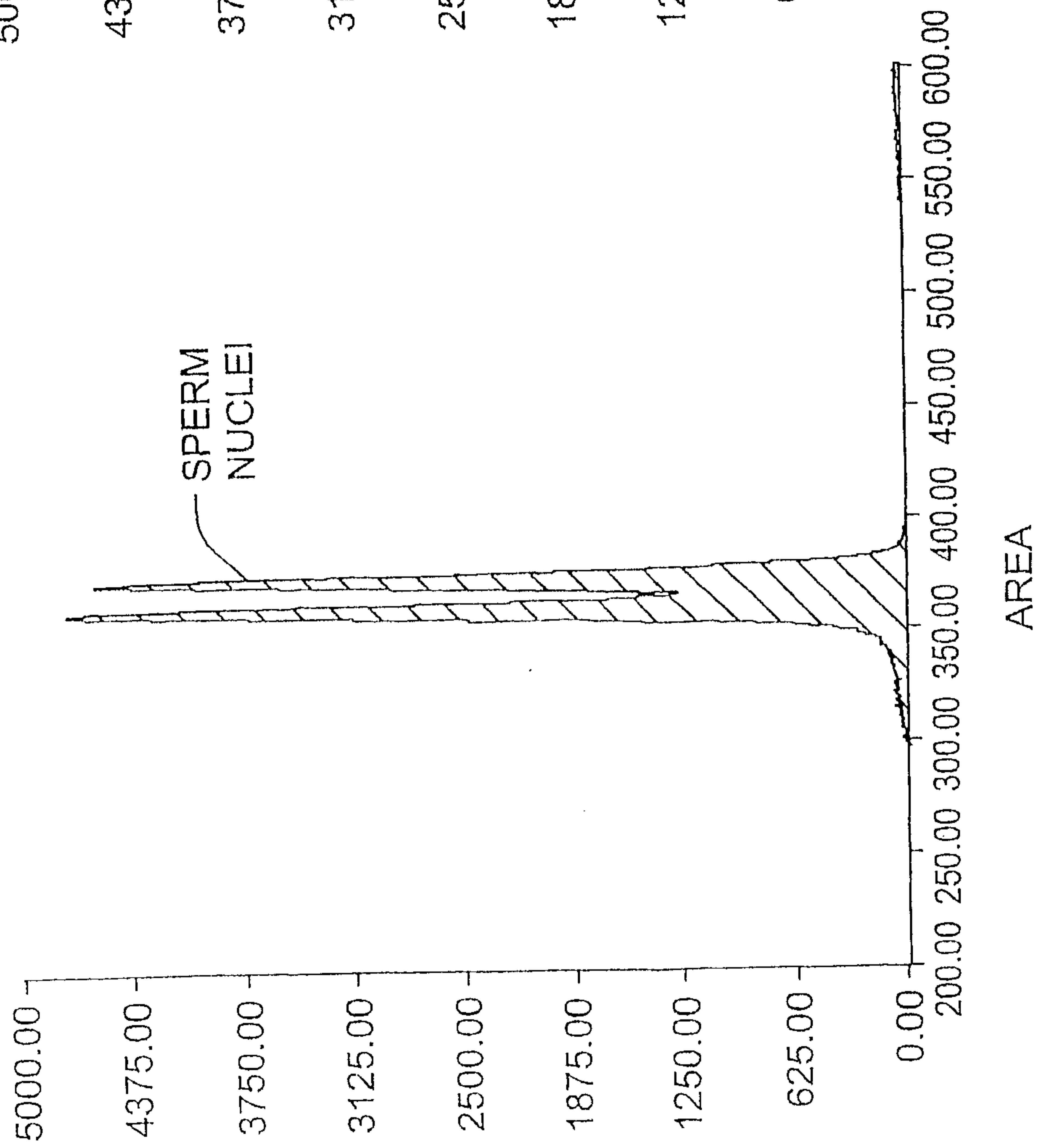


FIG. 59

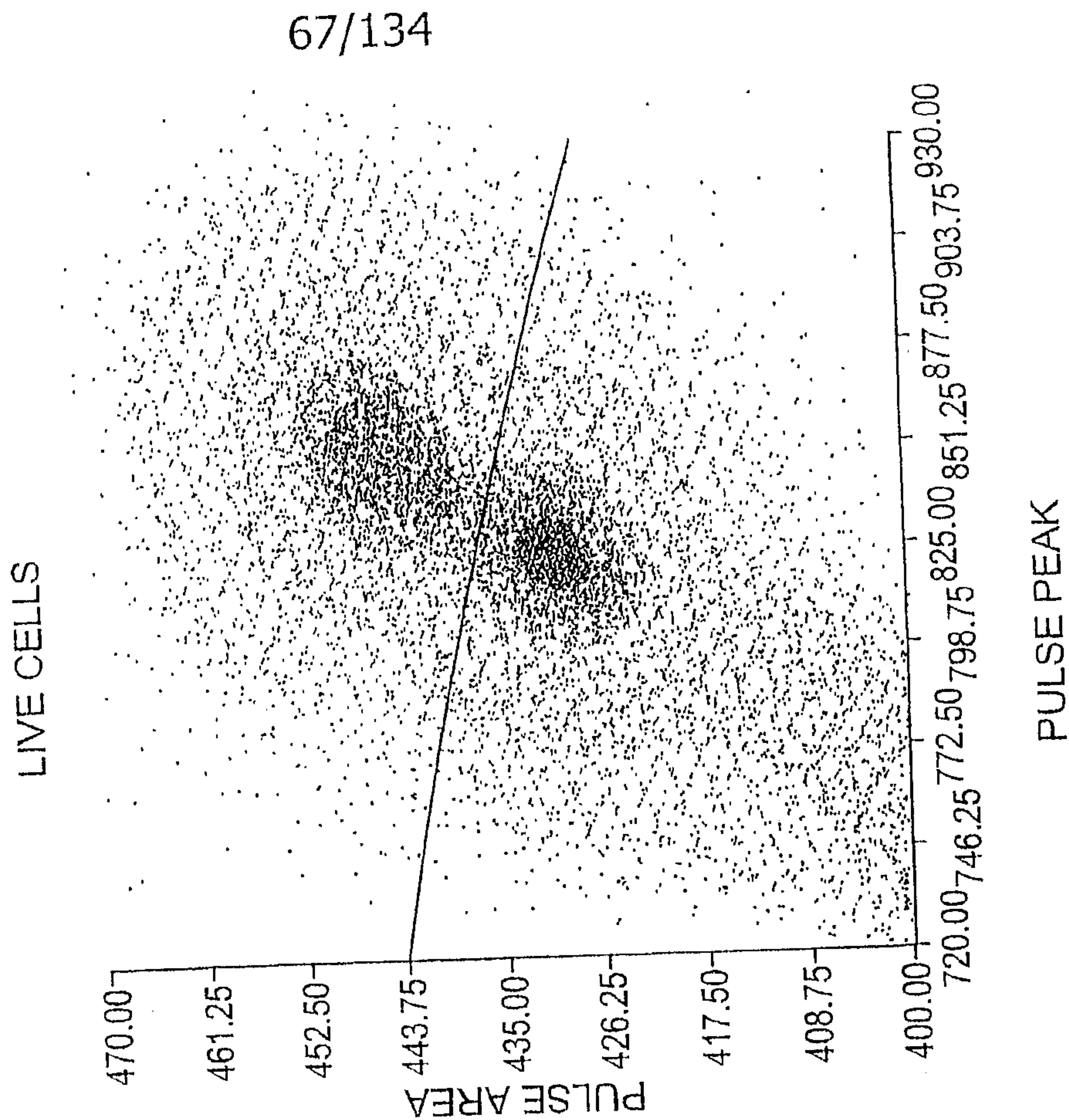


FIG. 58

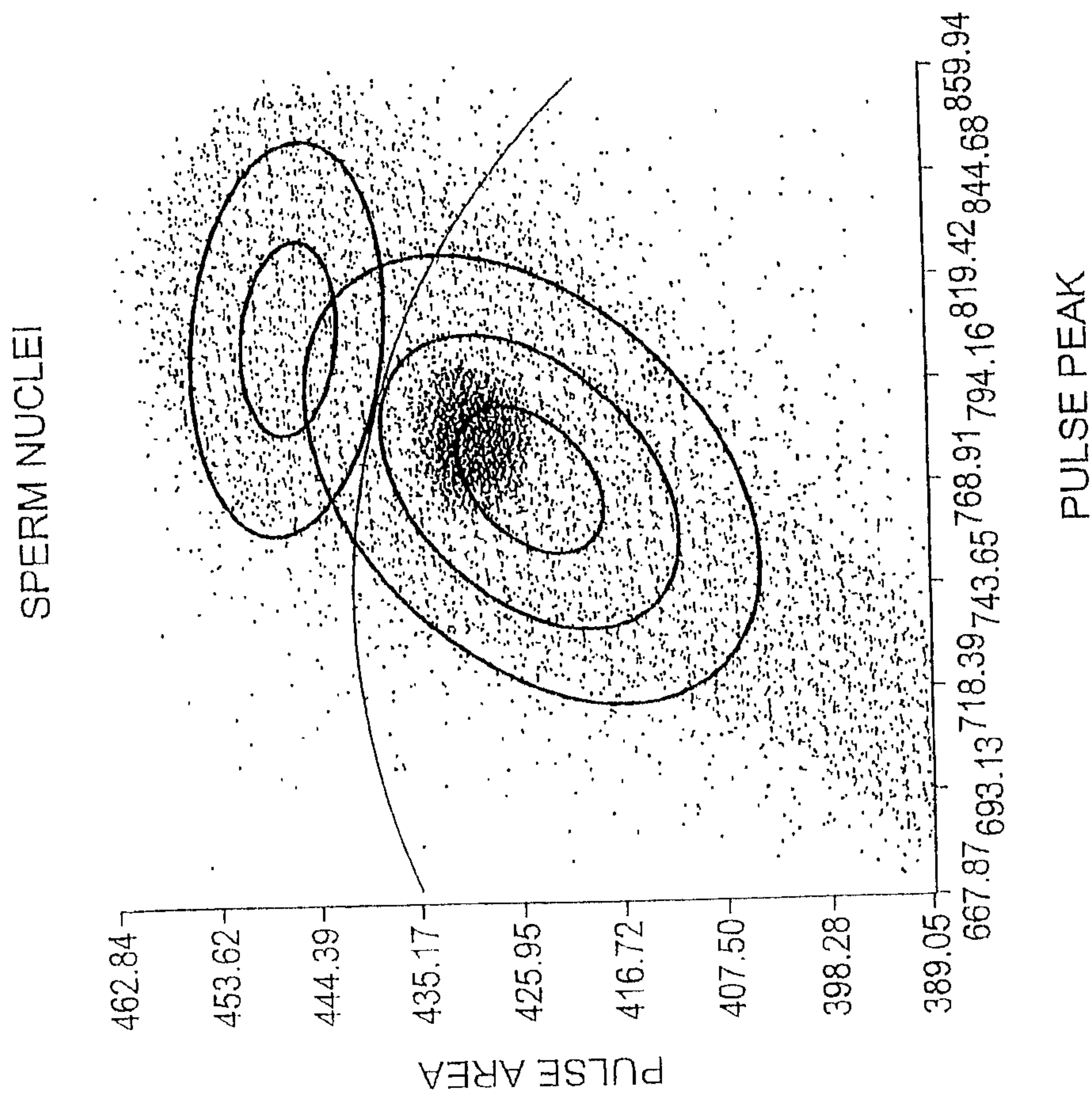


FIG. 60

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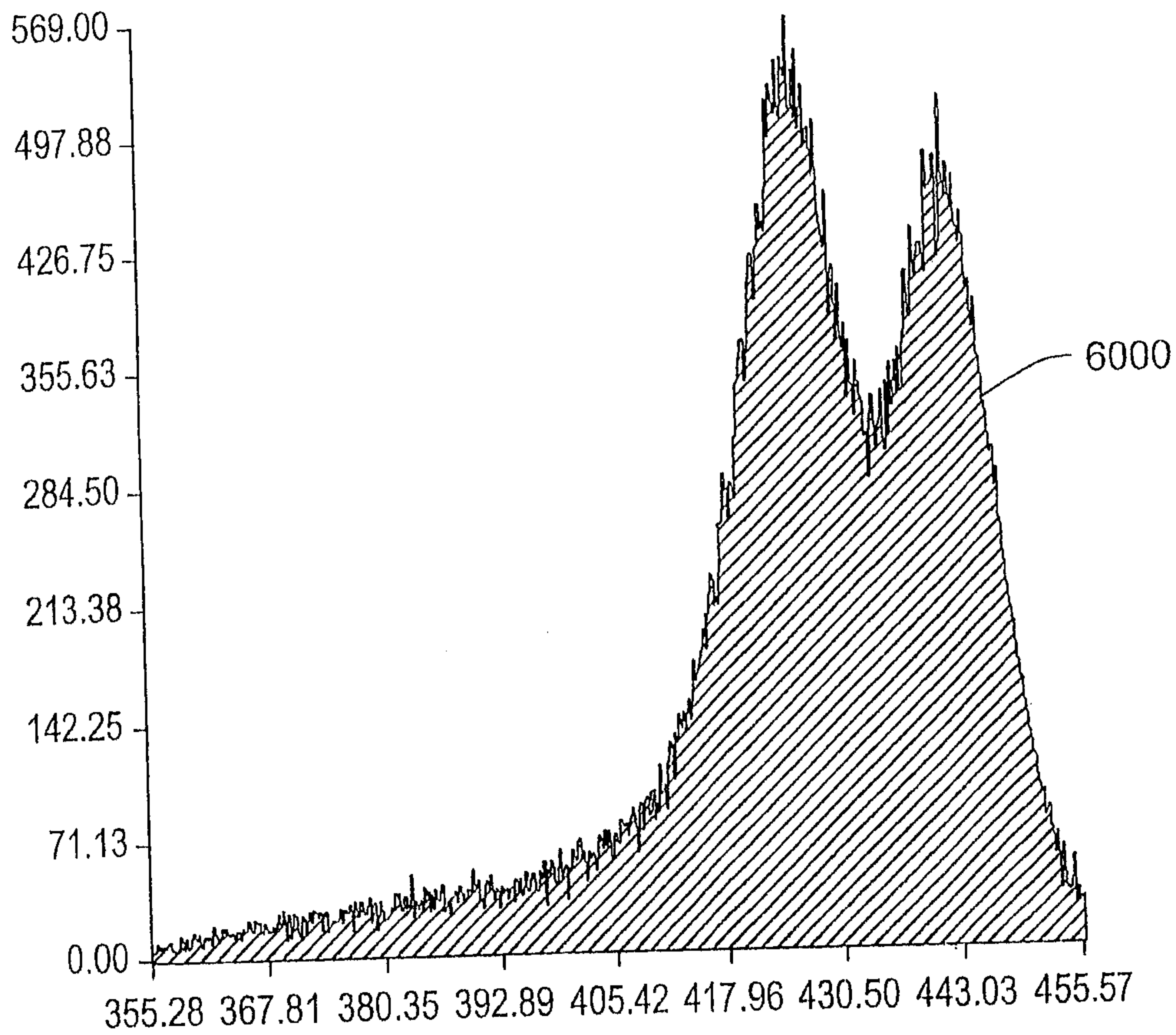


FIG. 61

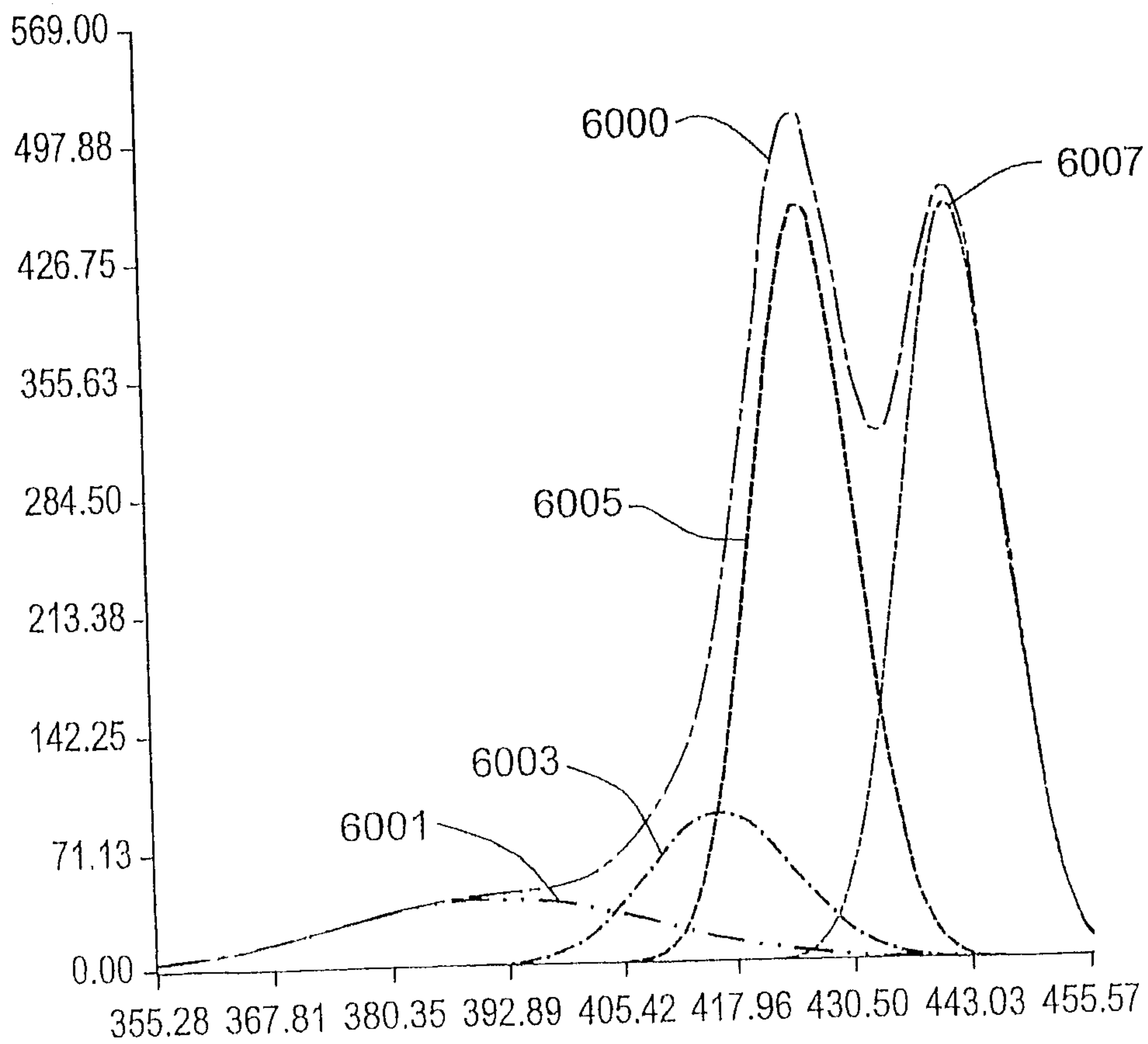


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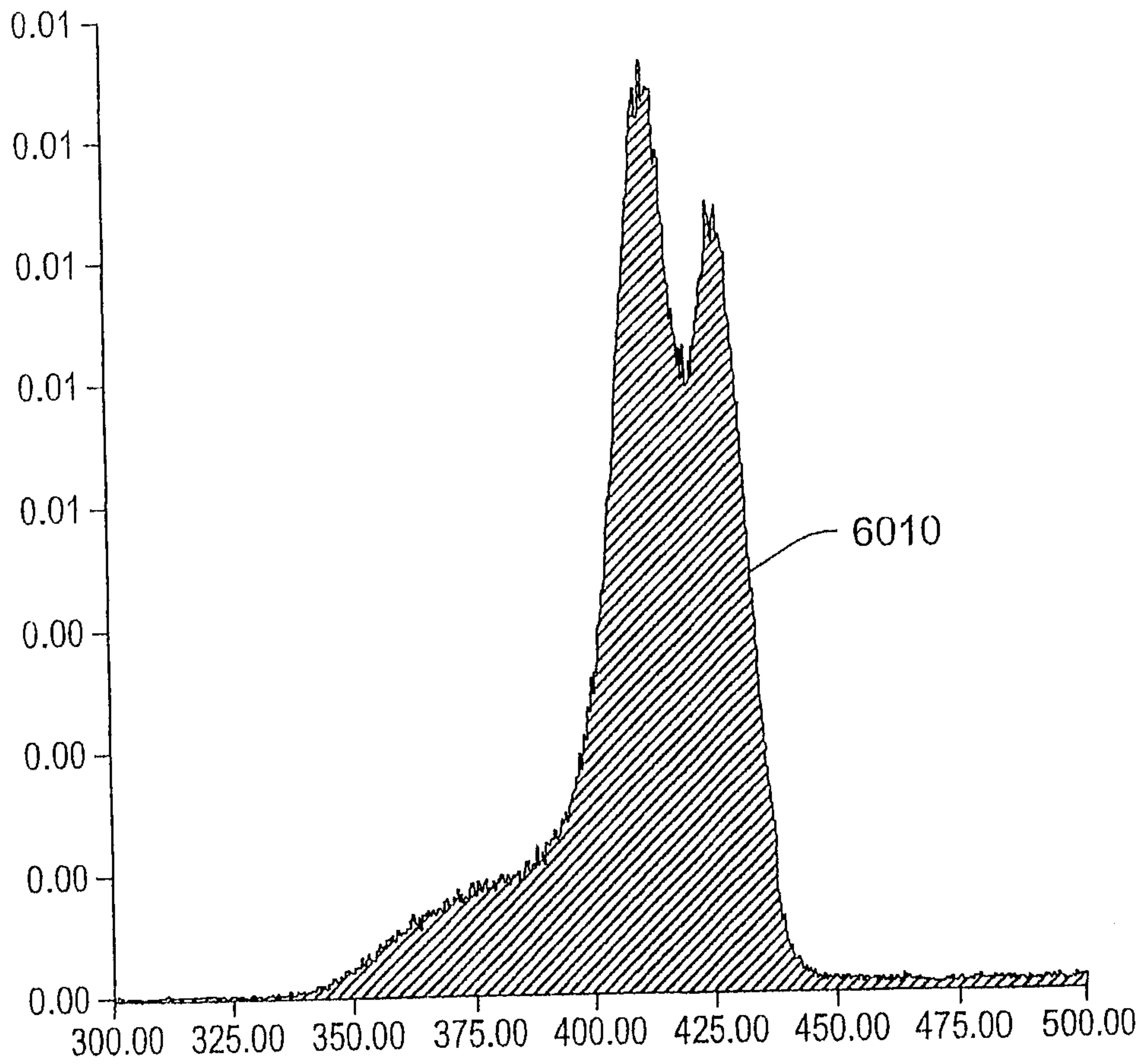


FIG. 63

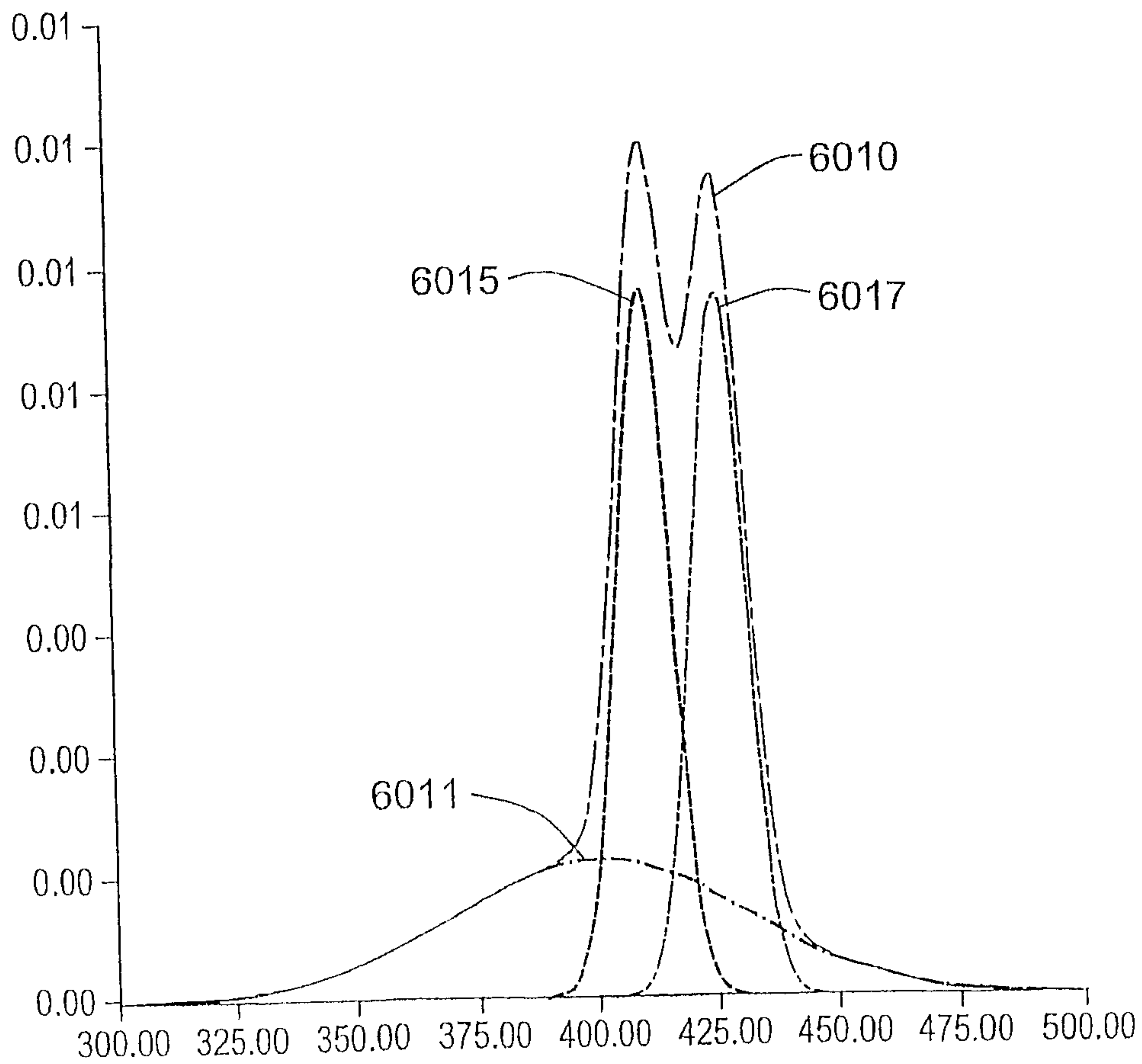
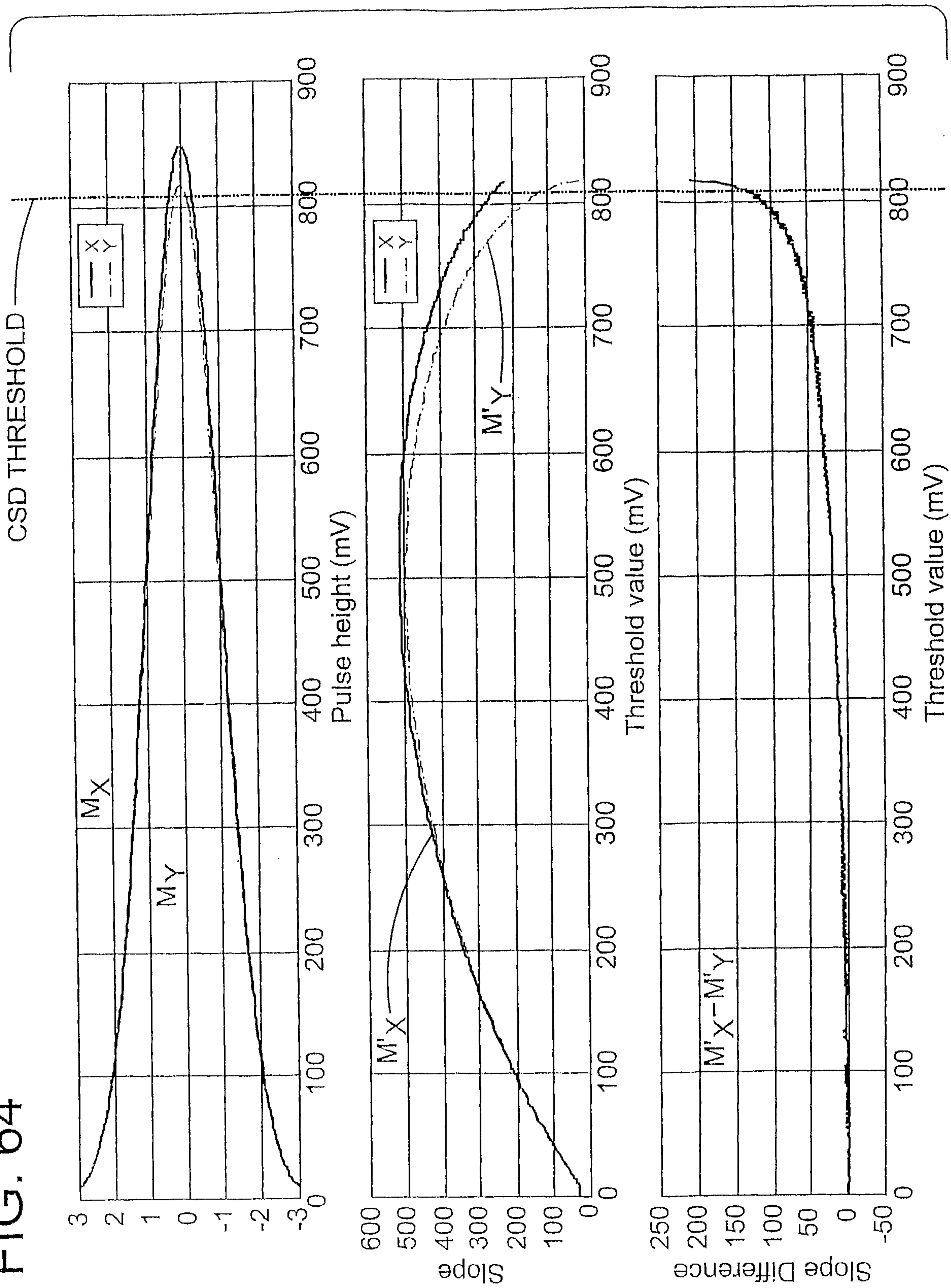
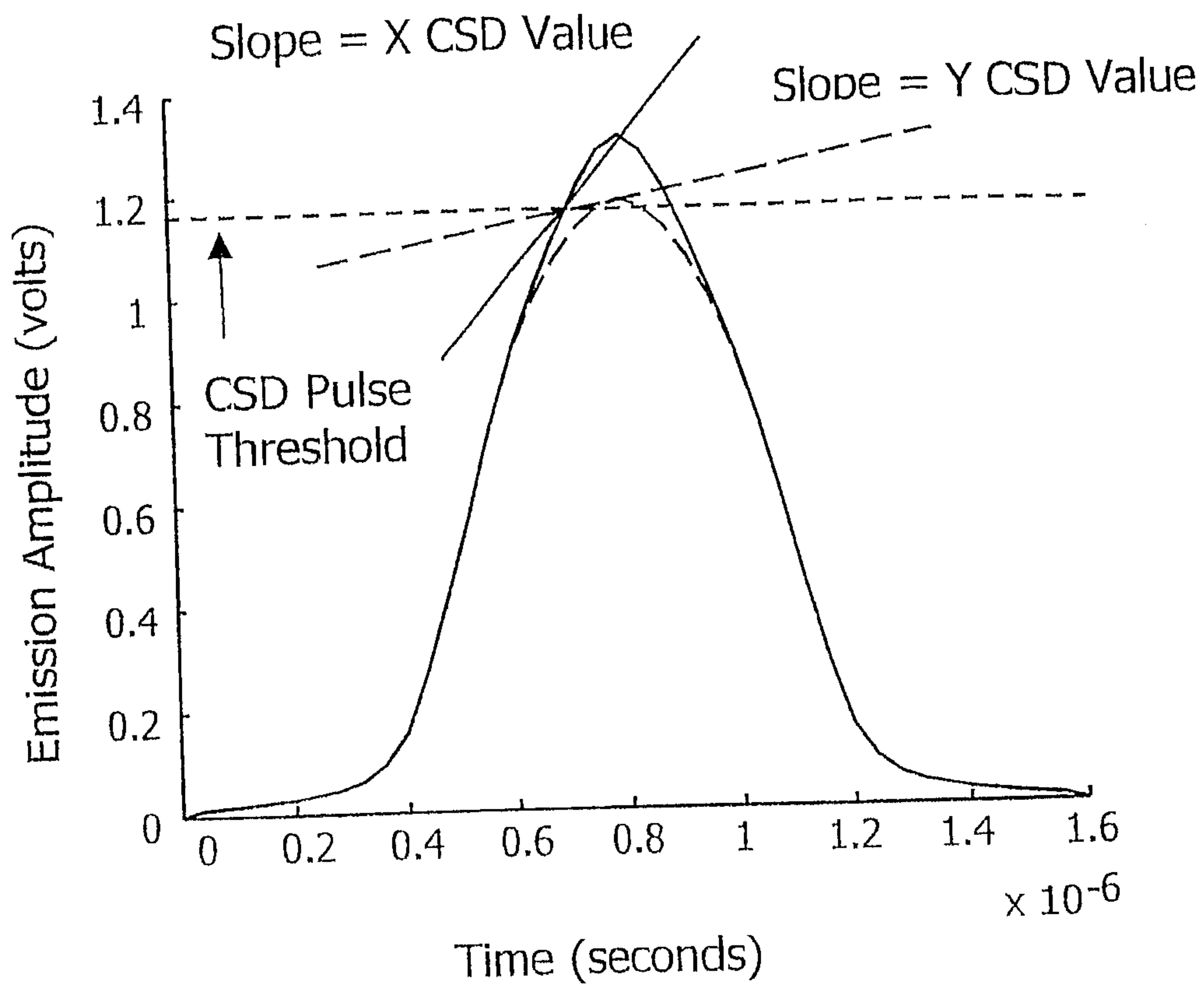


FIG. 64



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FIG. 67

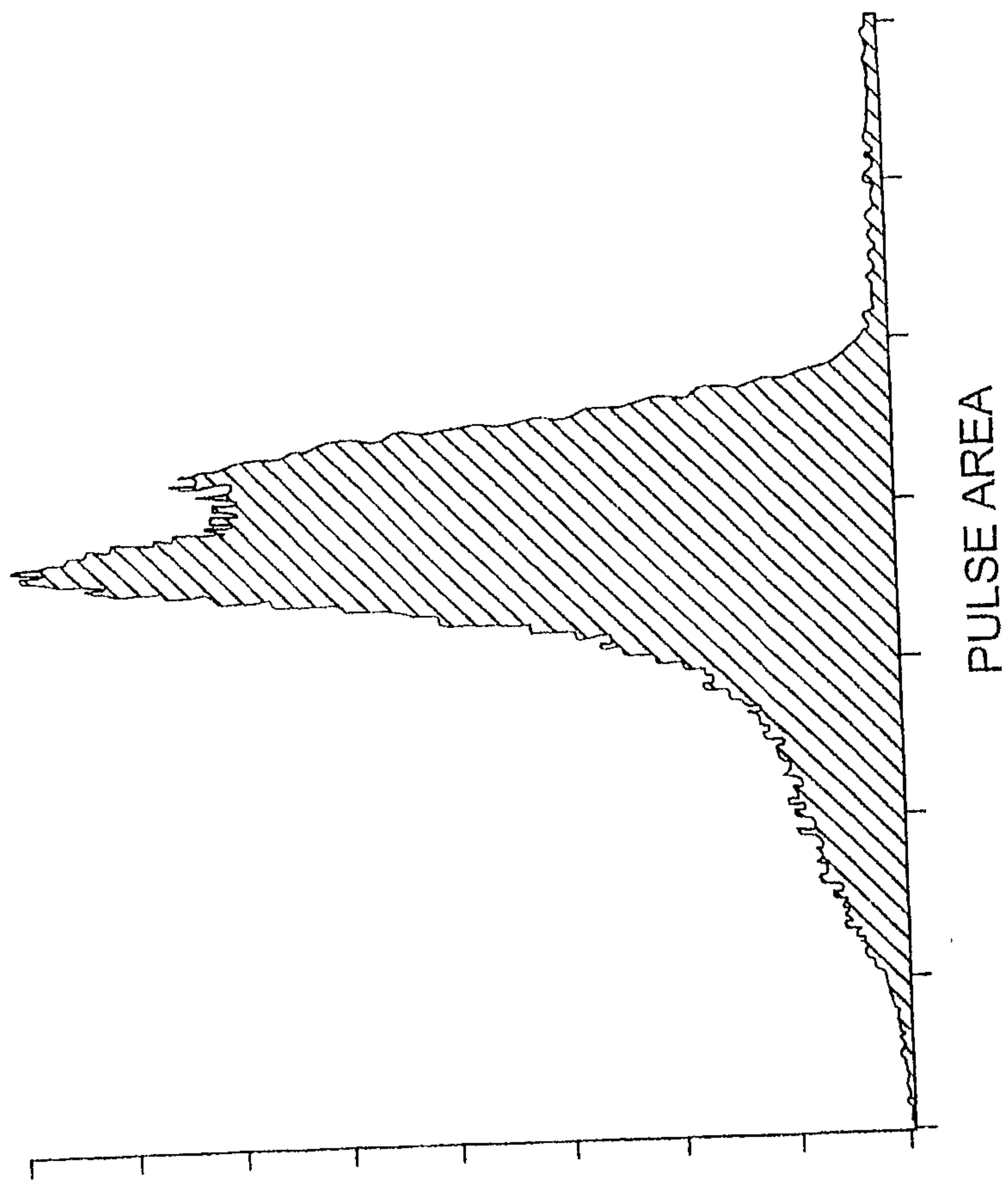


FIG. 66

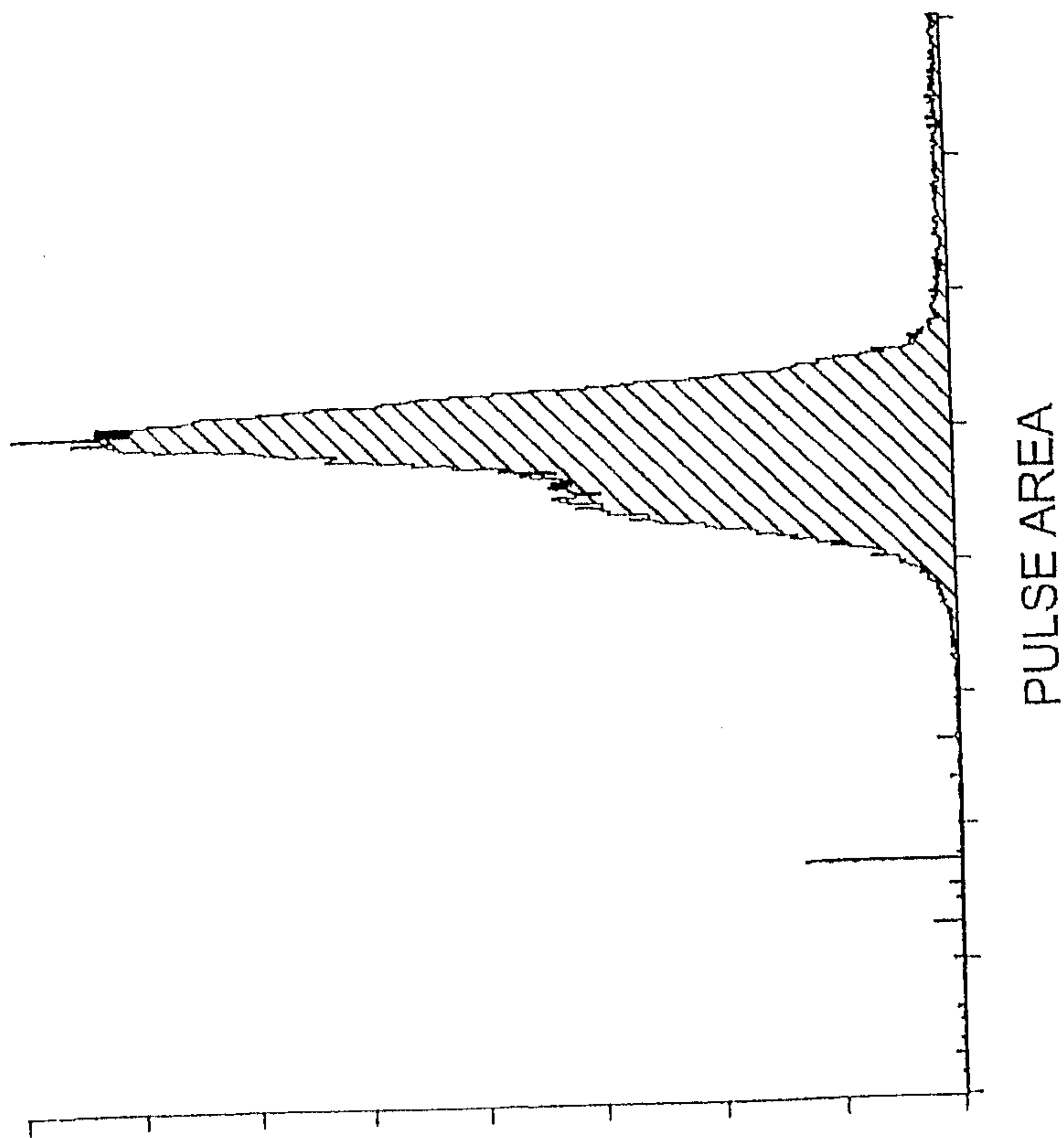


FIG. 68

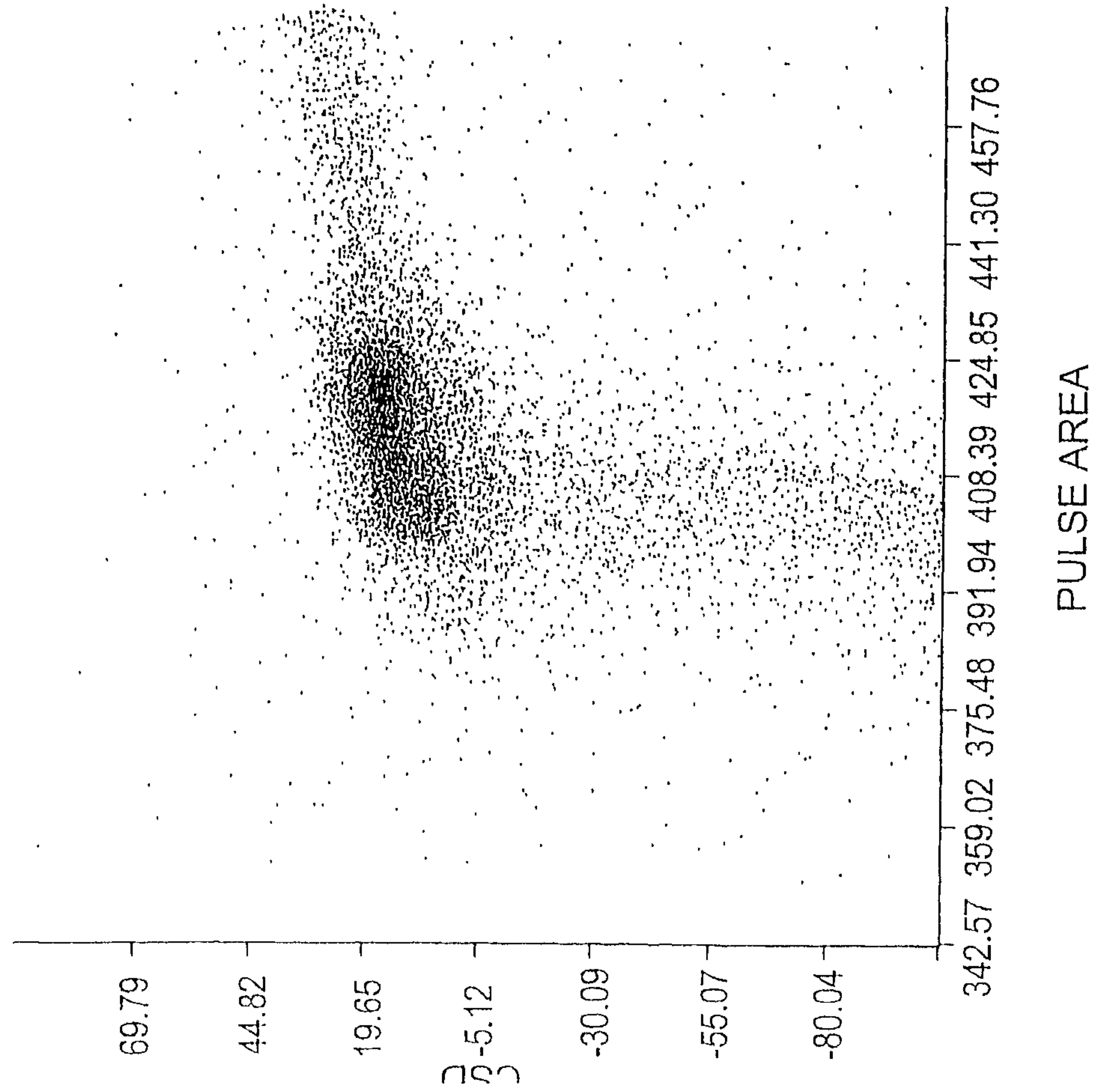
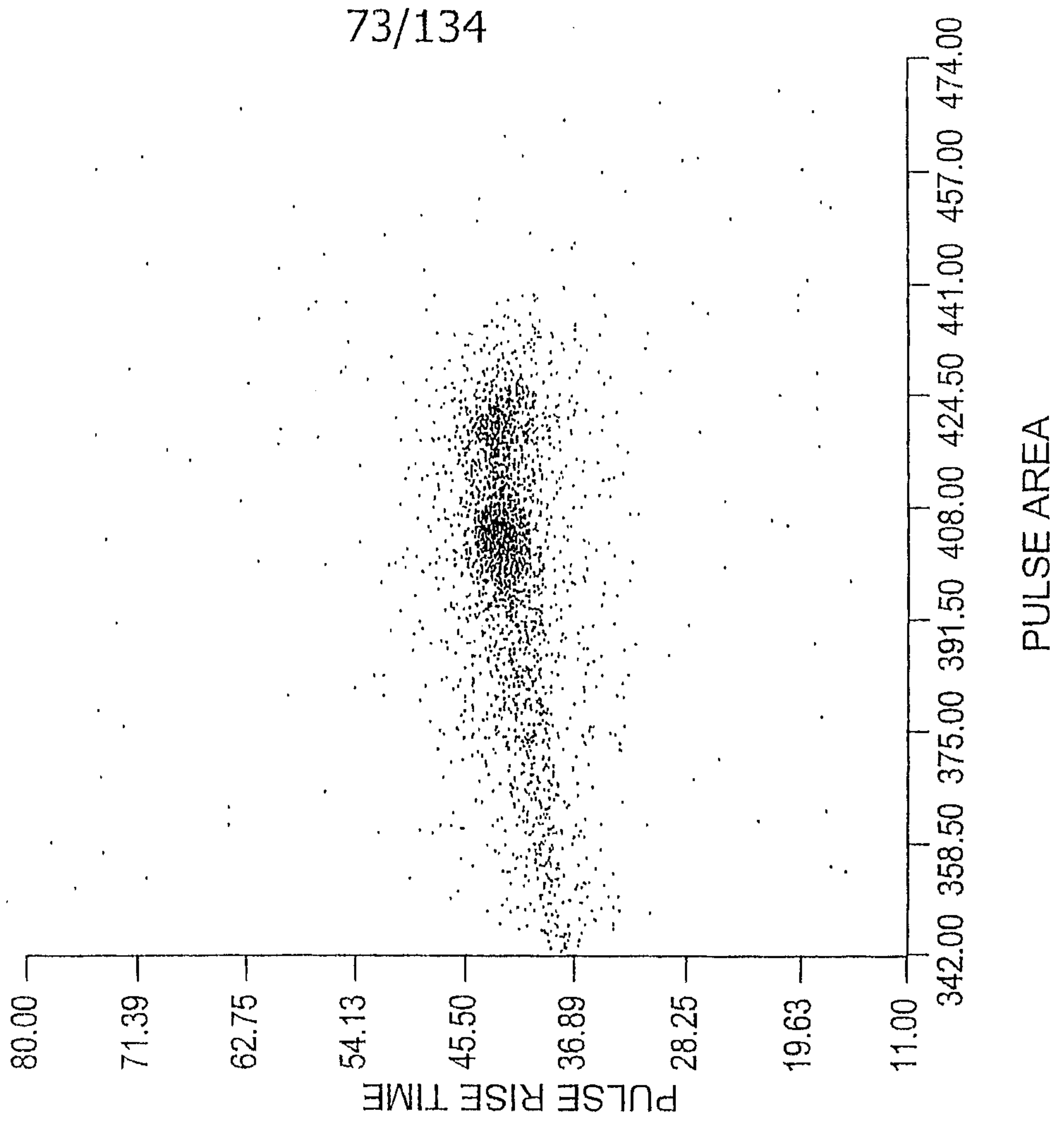
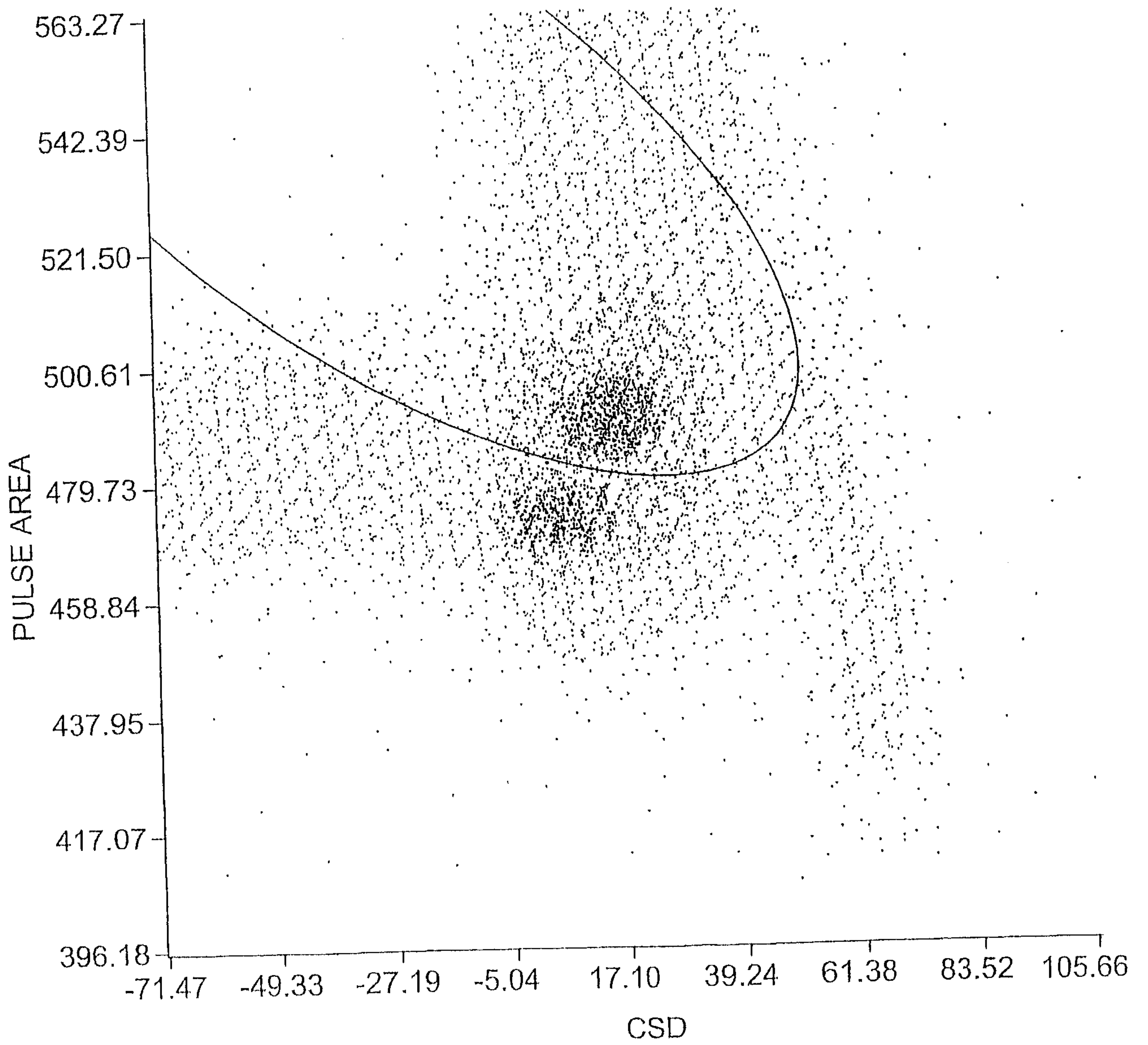


FIG. 69



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FIG. 70



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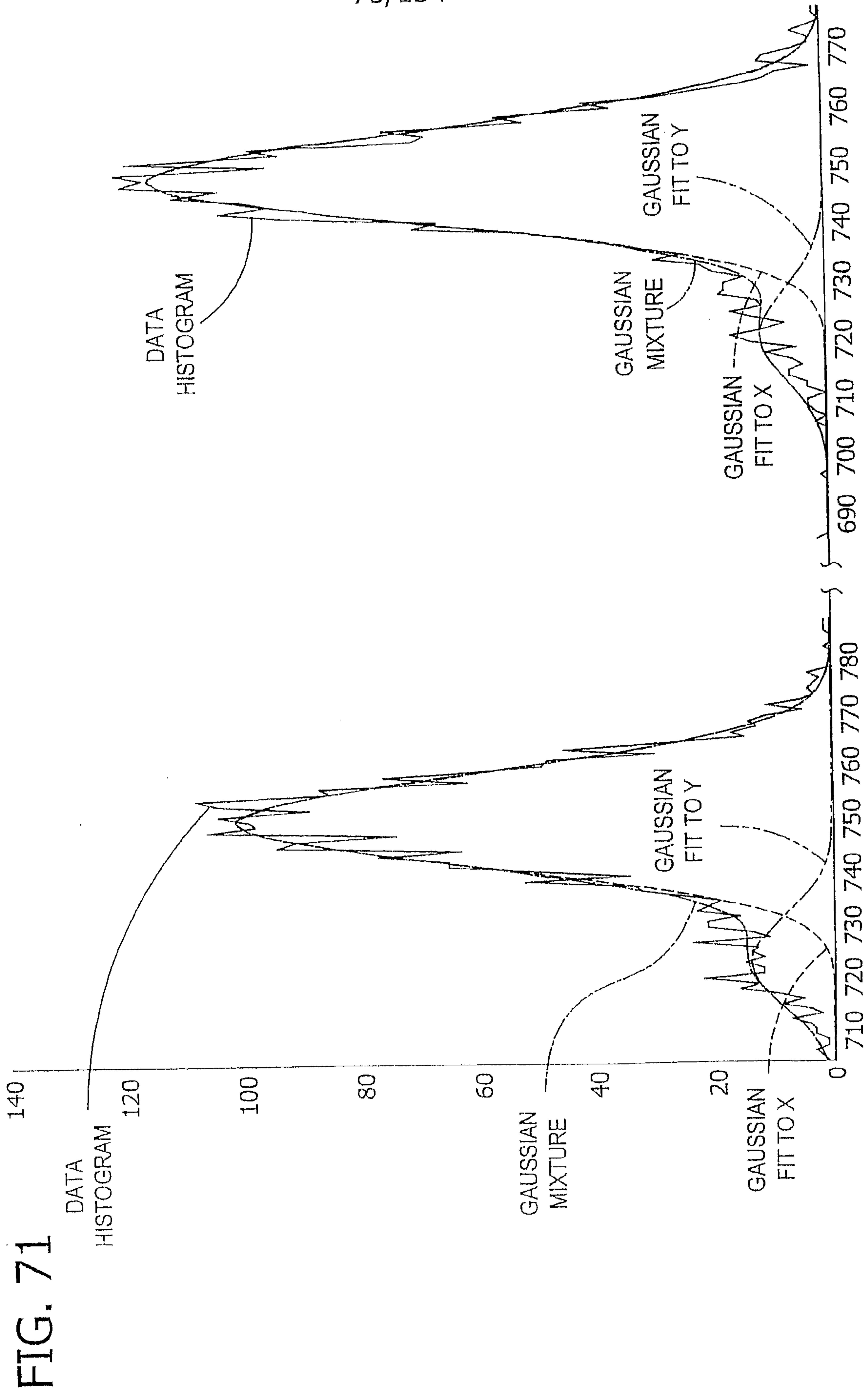
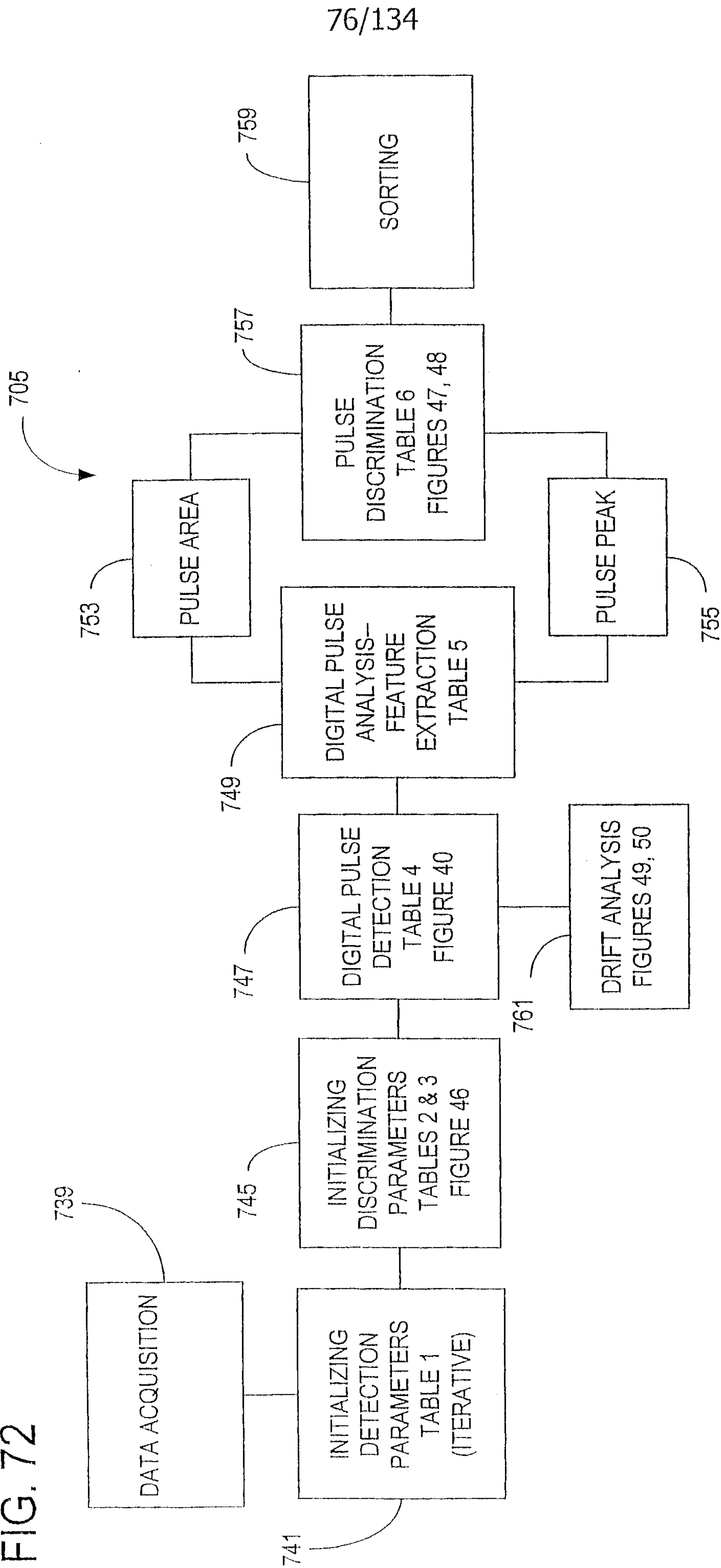


FIG. 71

FIG. 72



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FIG. 73

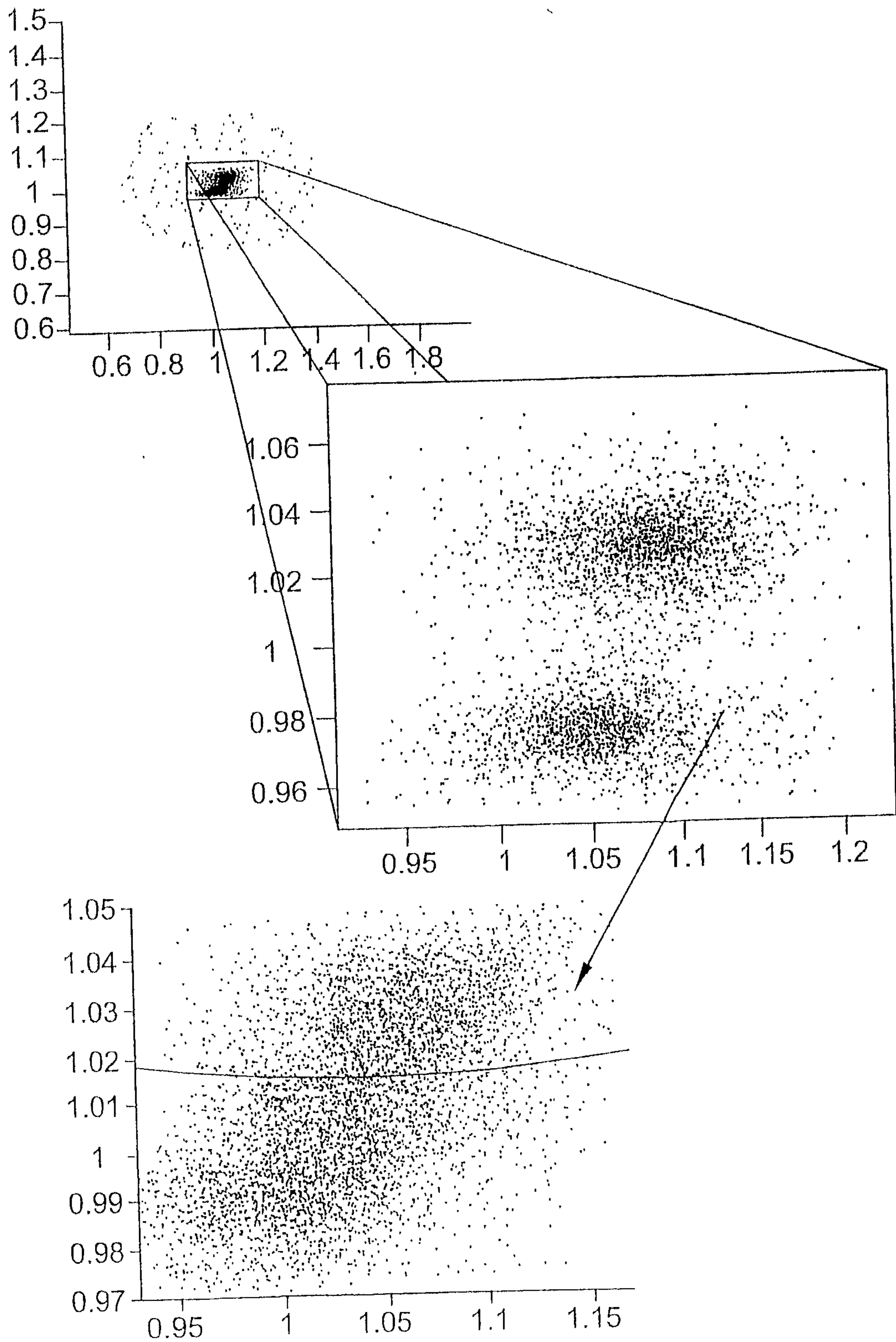
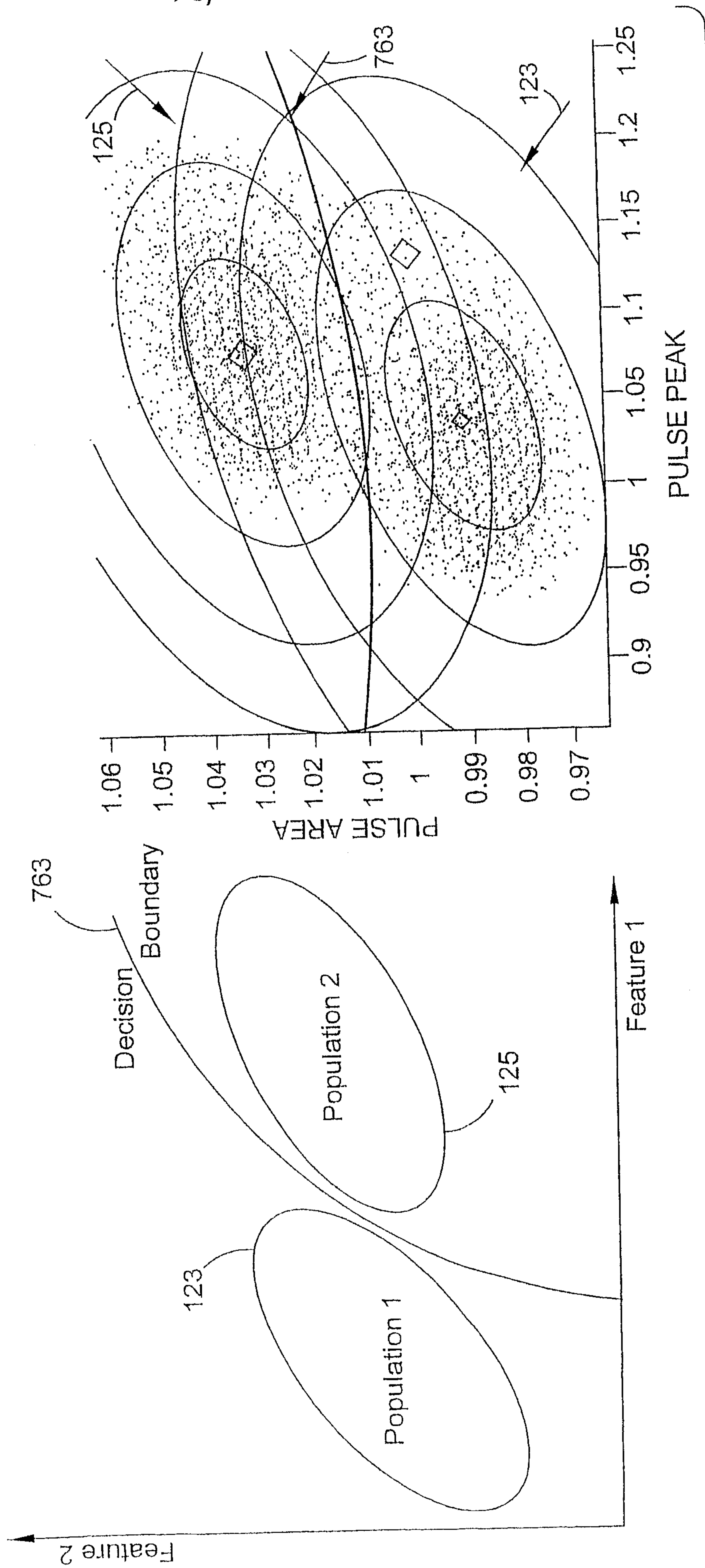


FIG. 74



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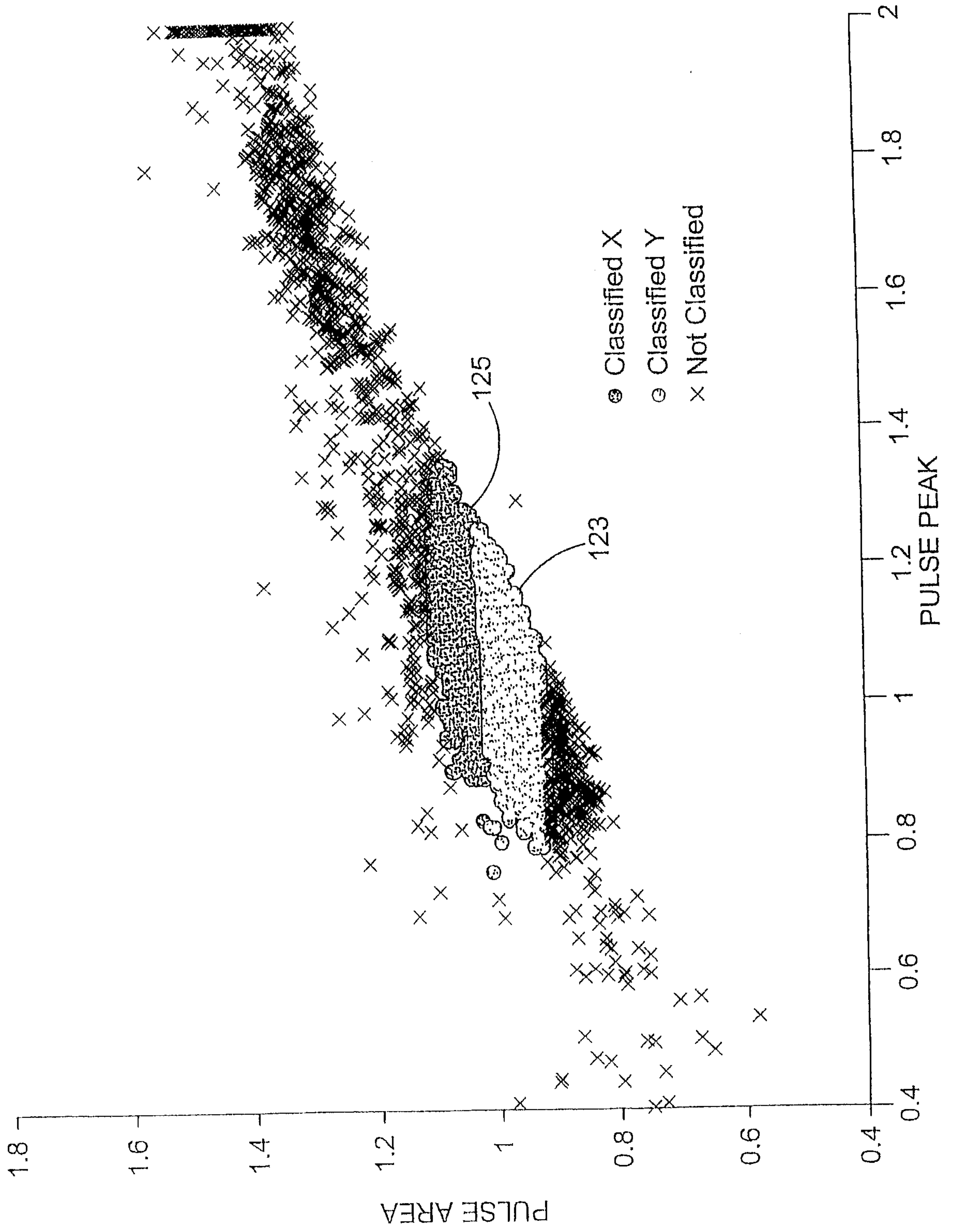
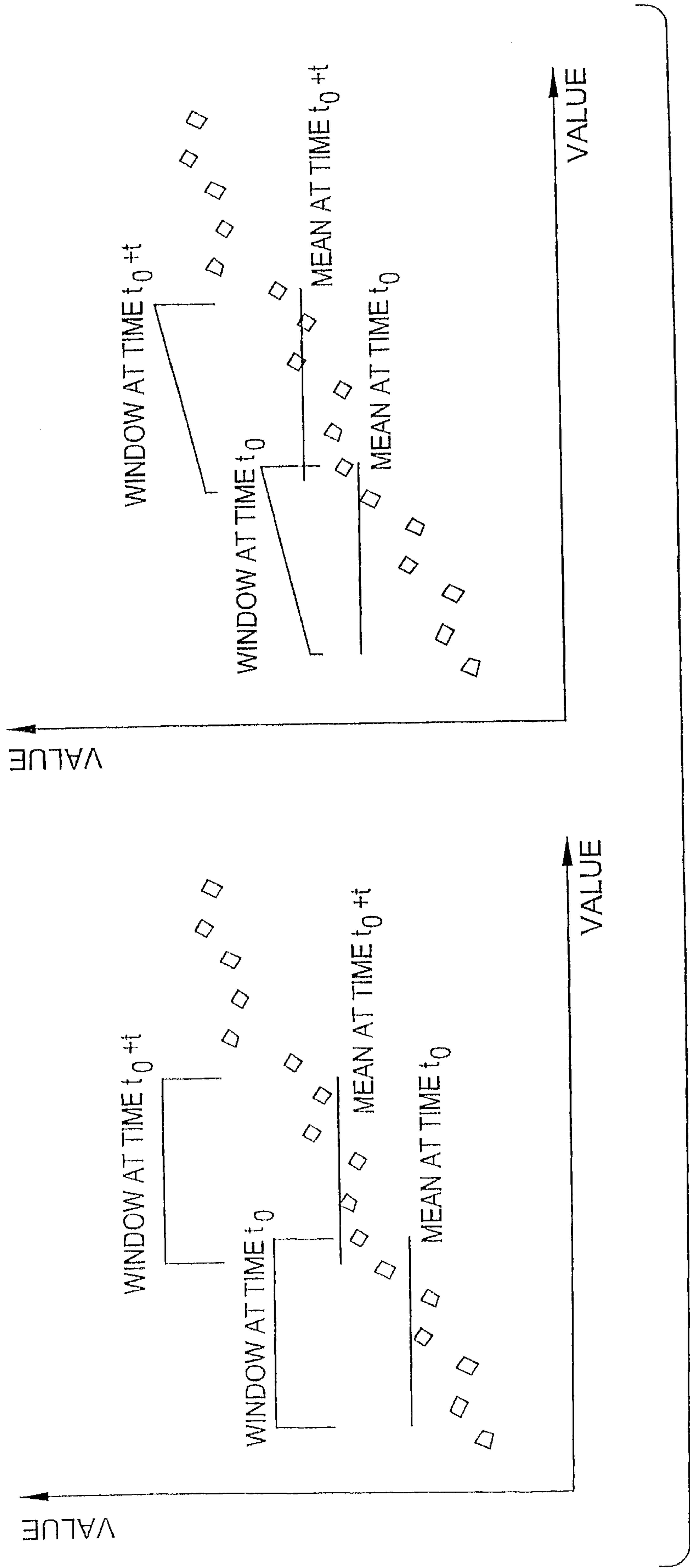
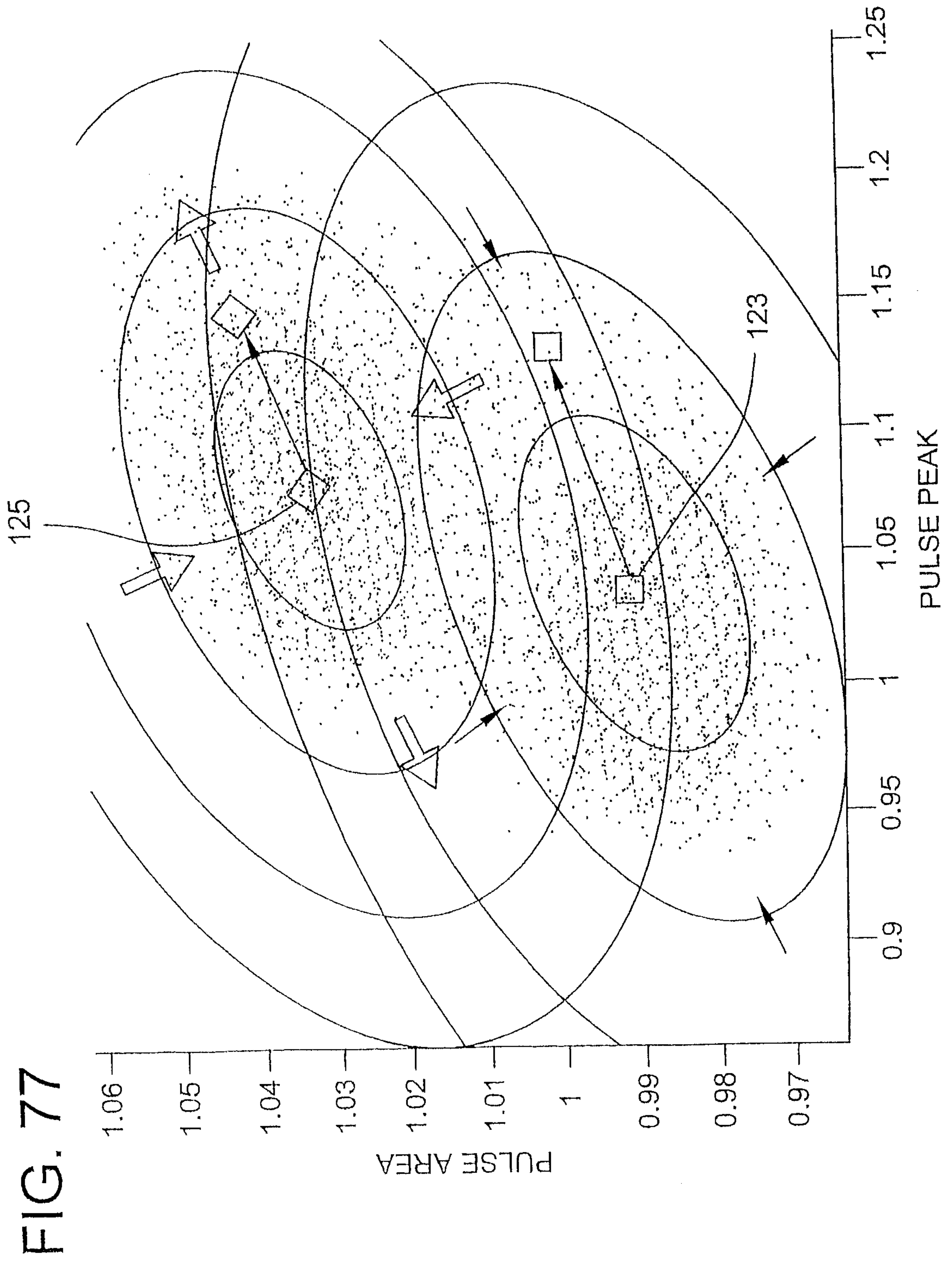


FIG. 75

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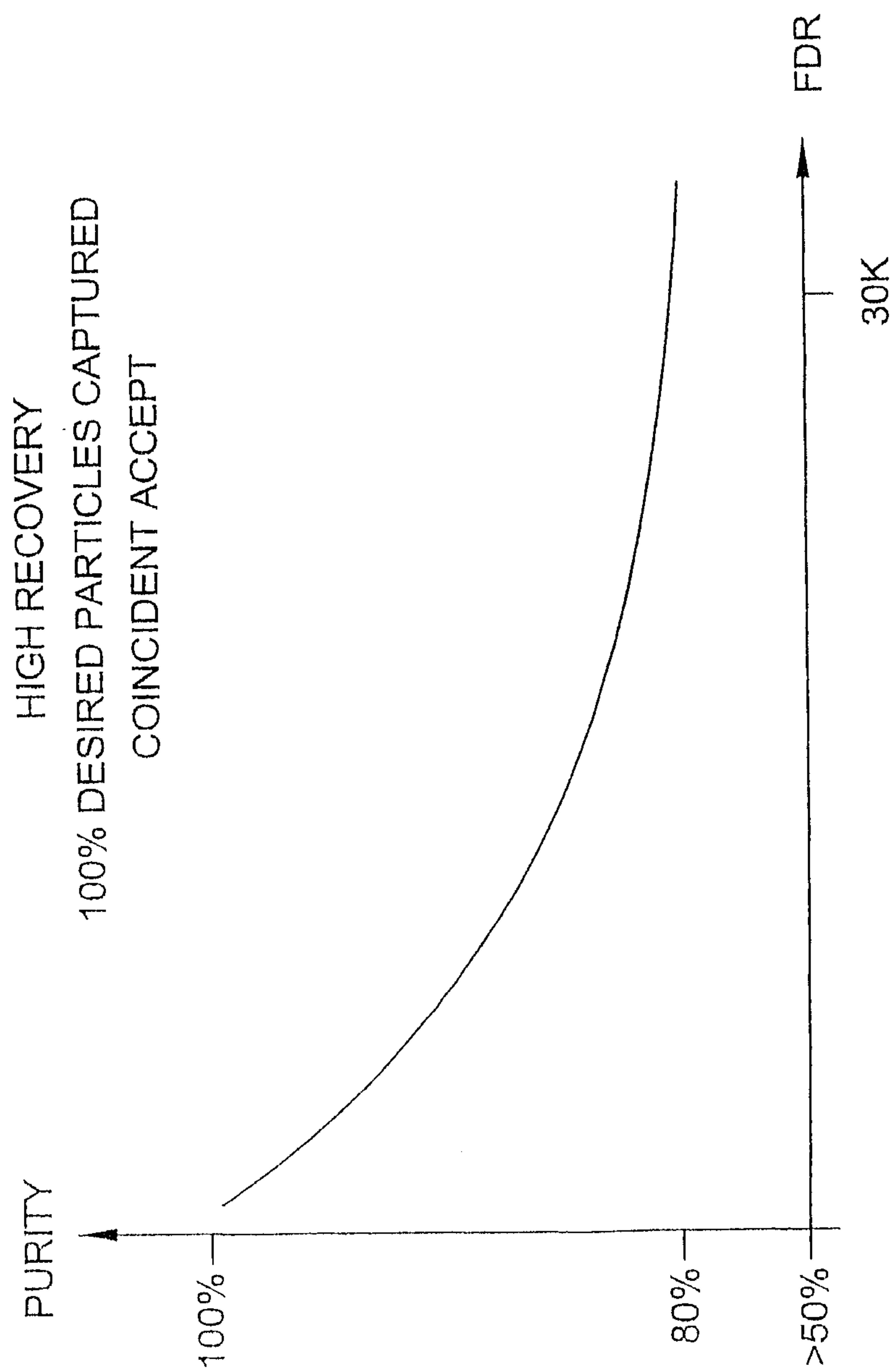
FIG. 76





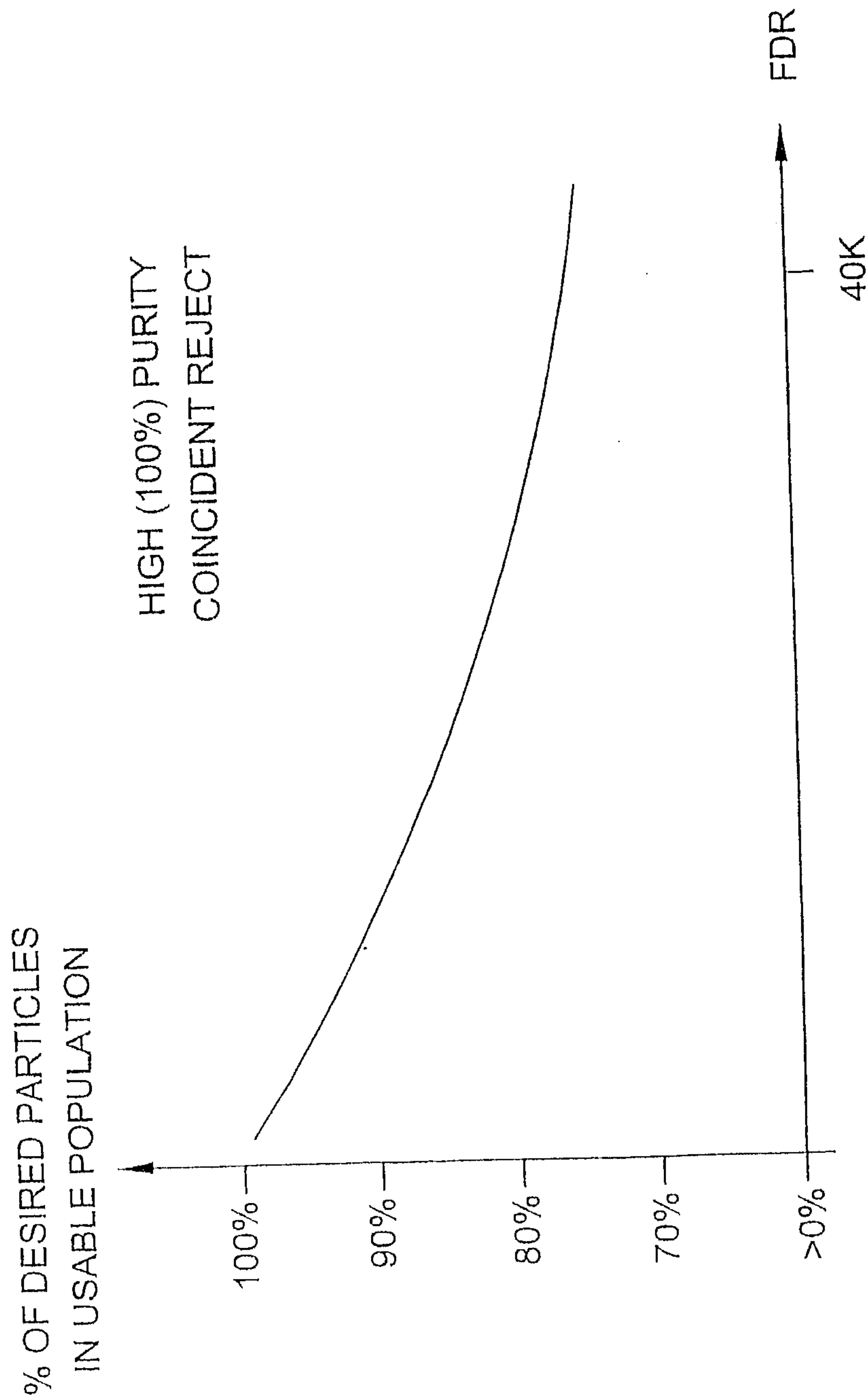
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FIG. 79



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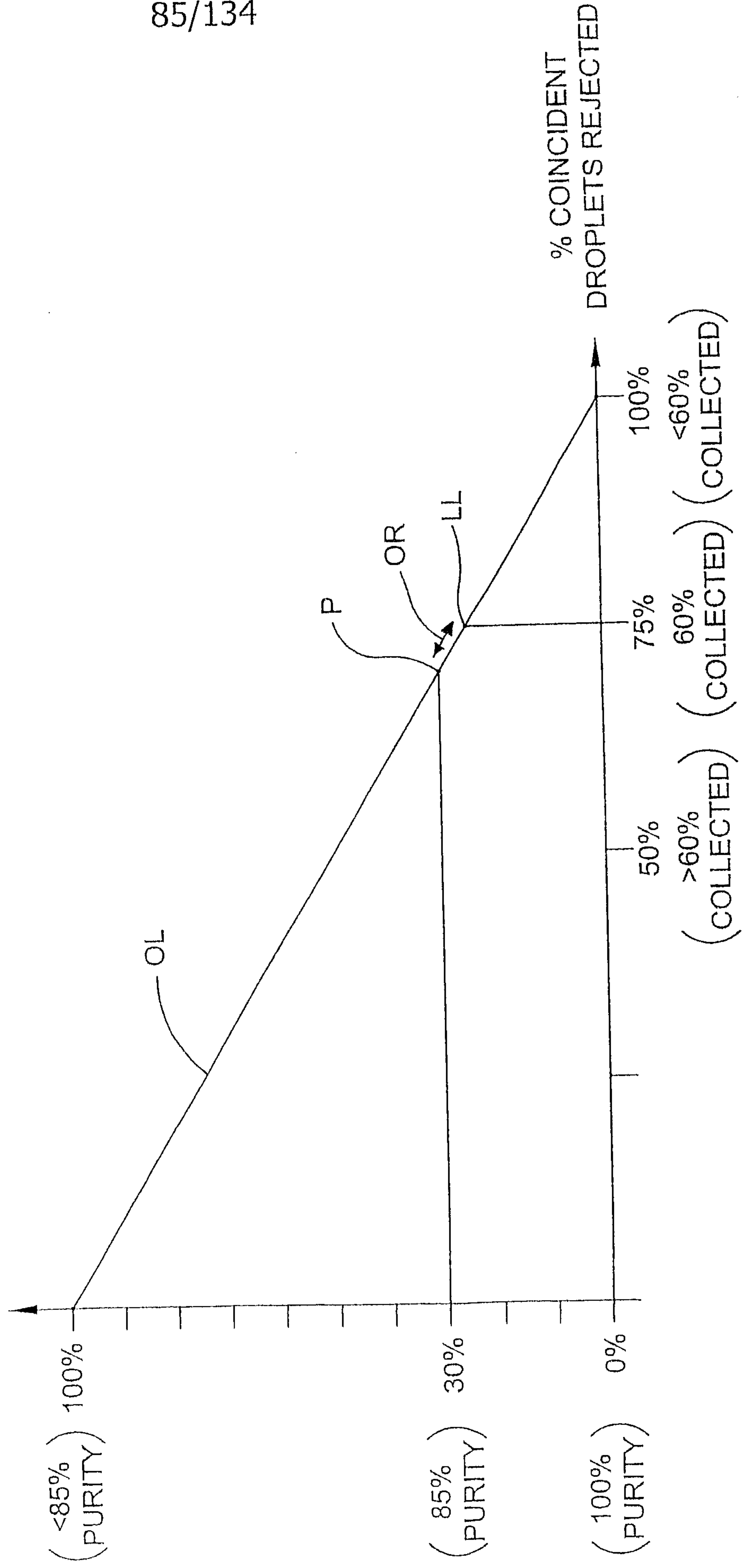
FIG. 80



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FIG. 81

% COINCIDENT DROPLETS ACCEPTED



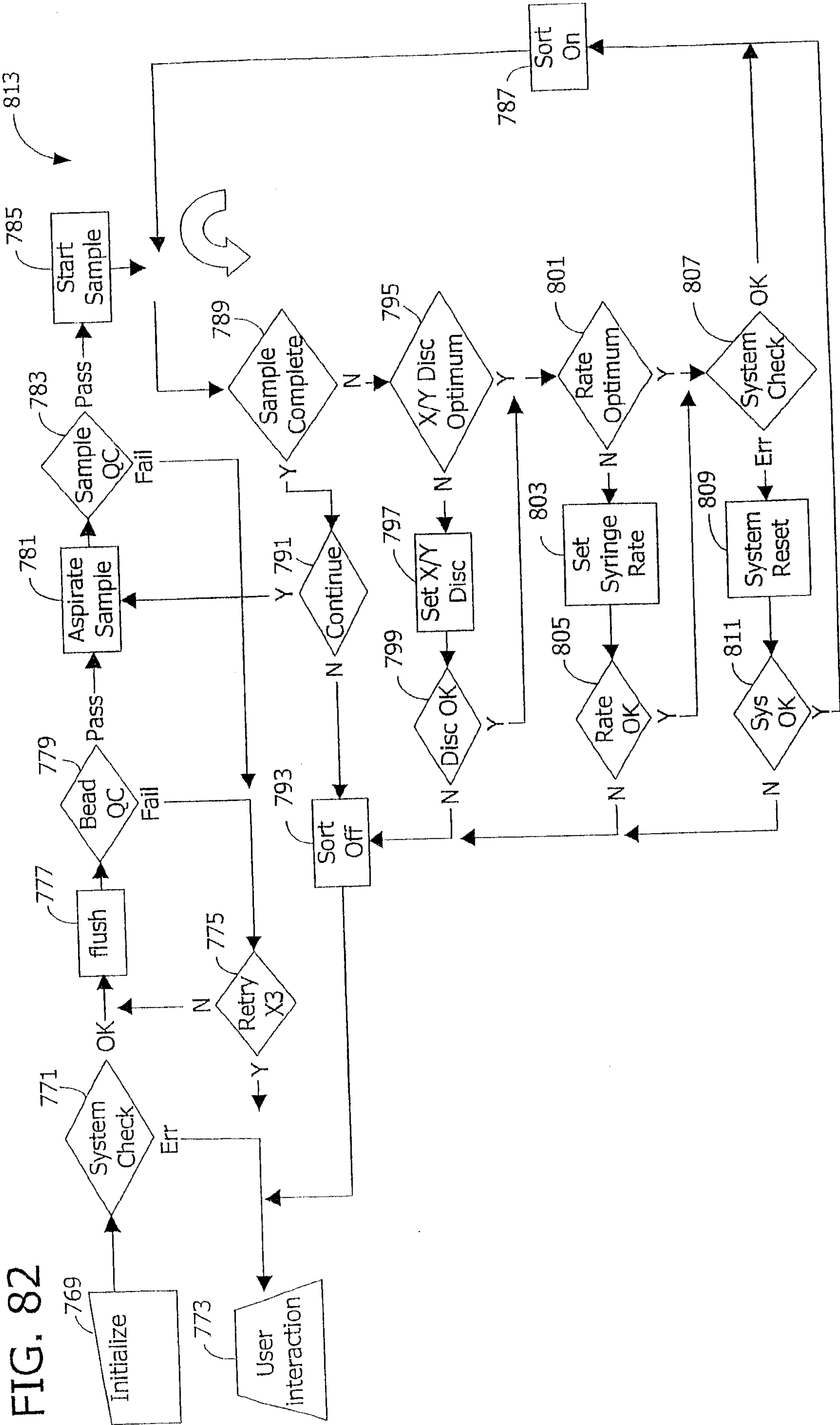
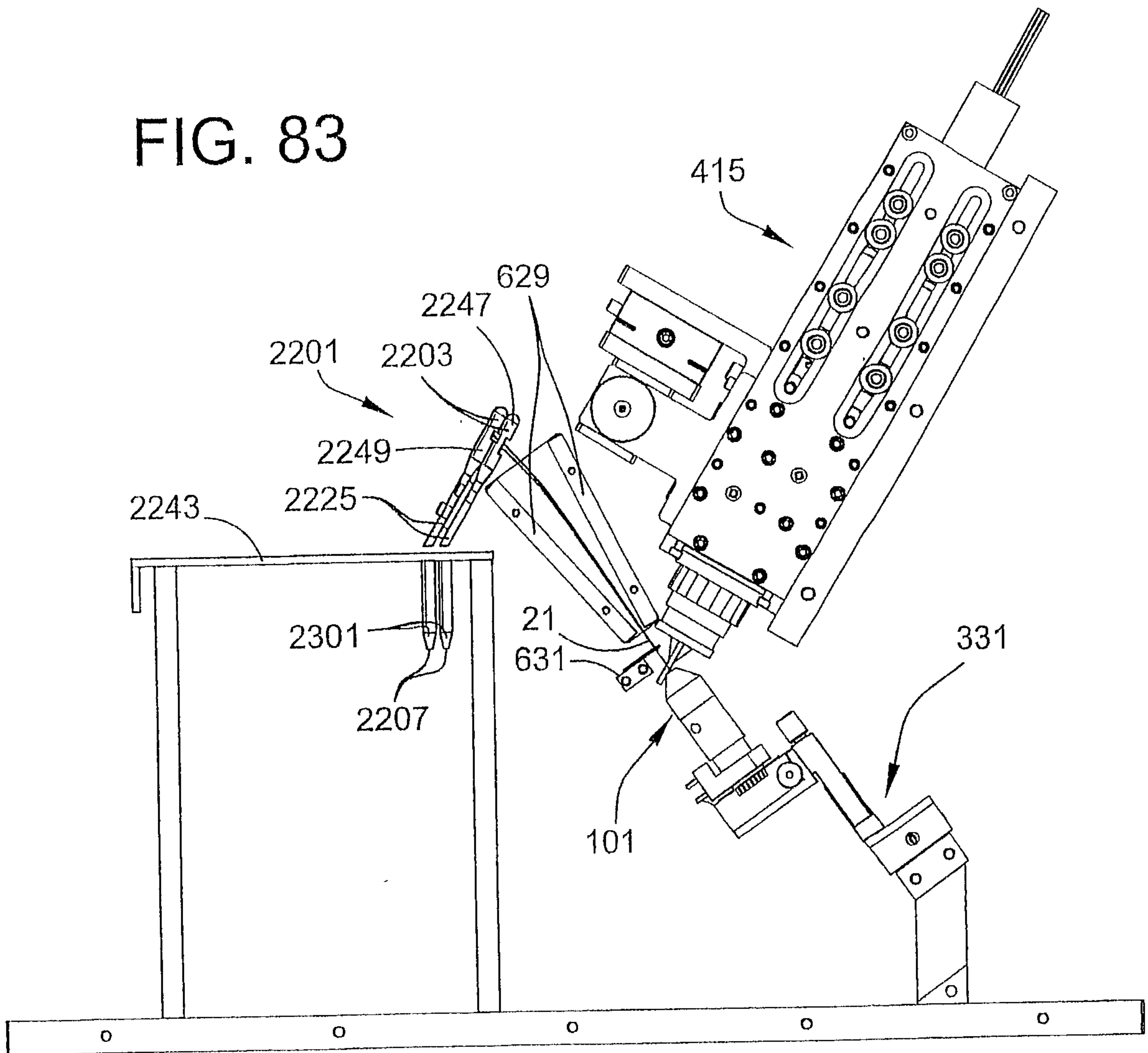


FIG. 82

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FIG. 83



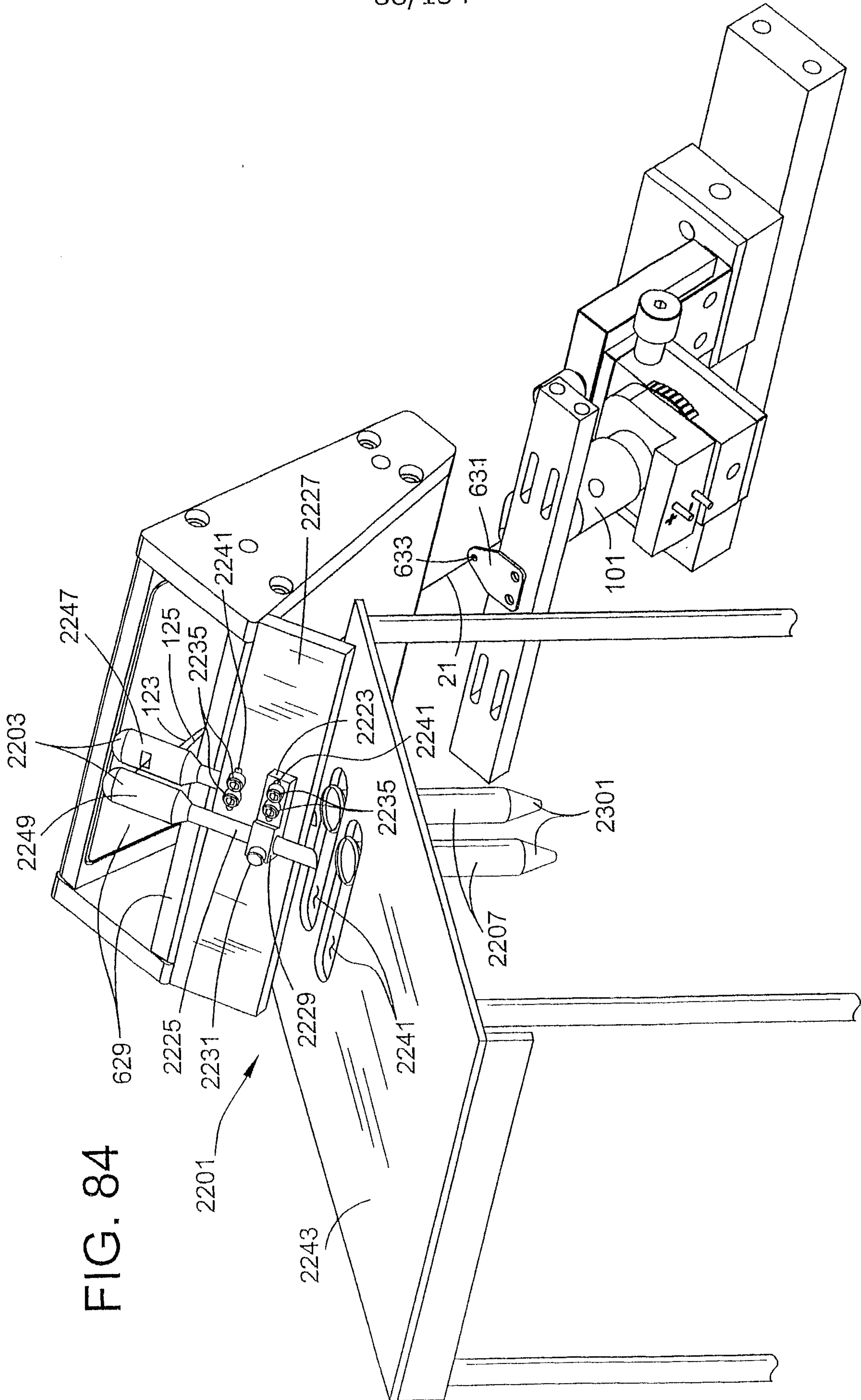


FIG. 84

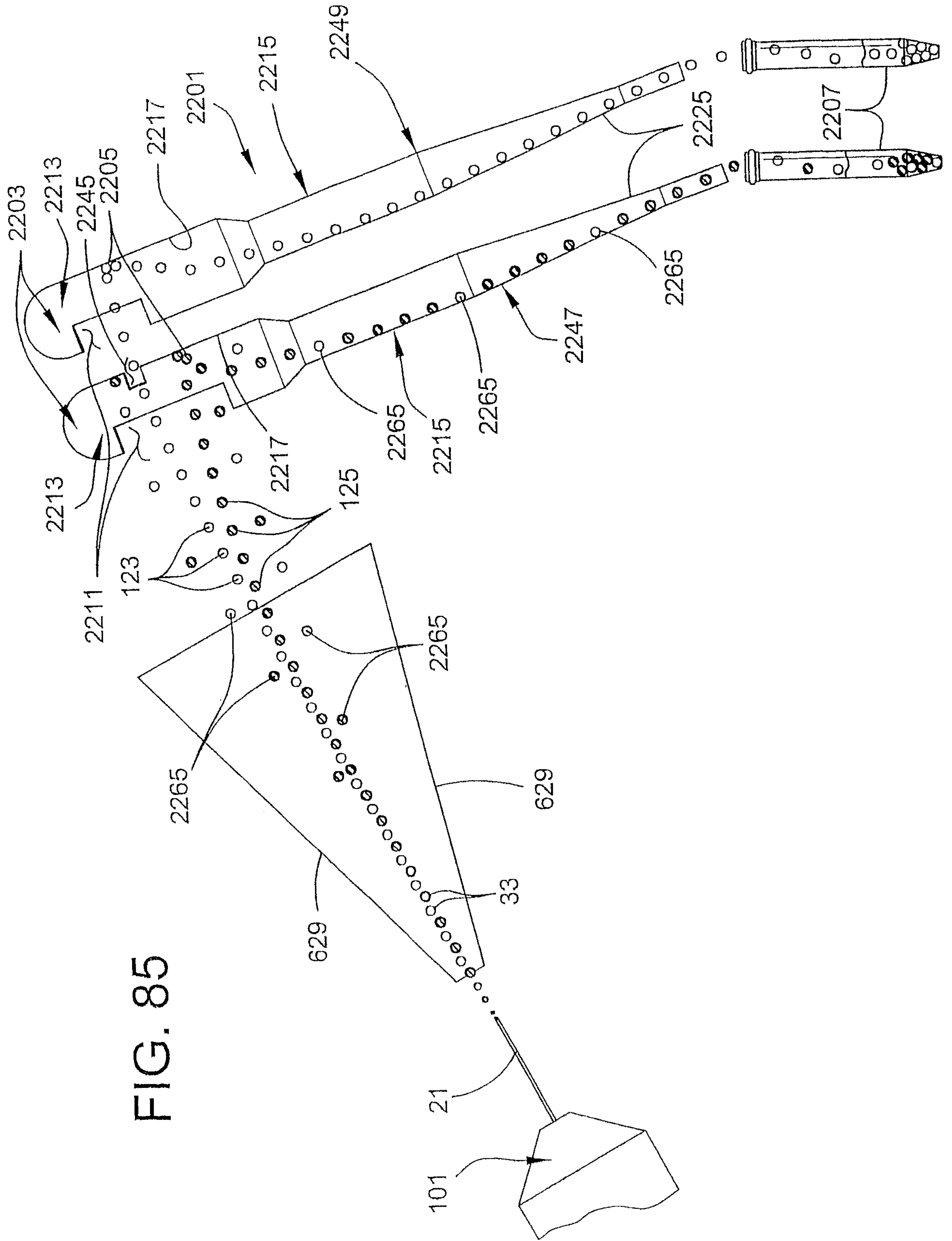


FIG. 85

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FIG. 86

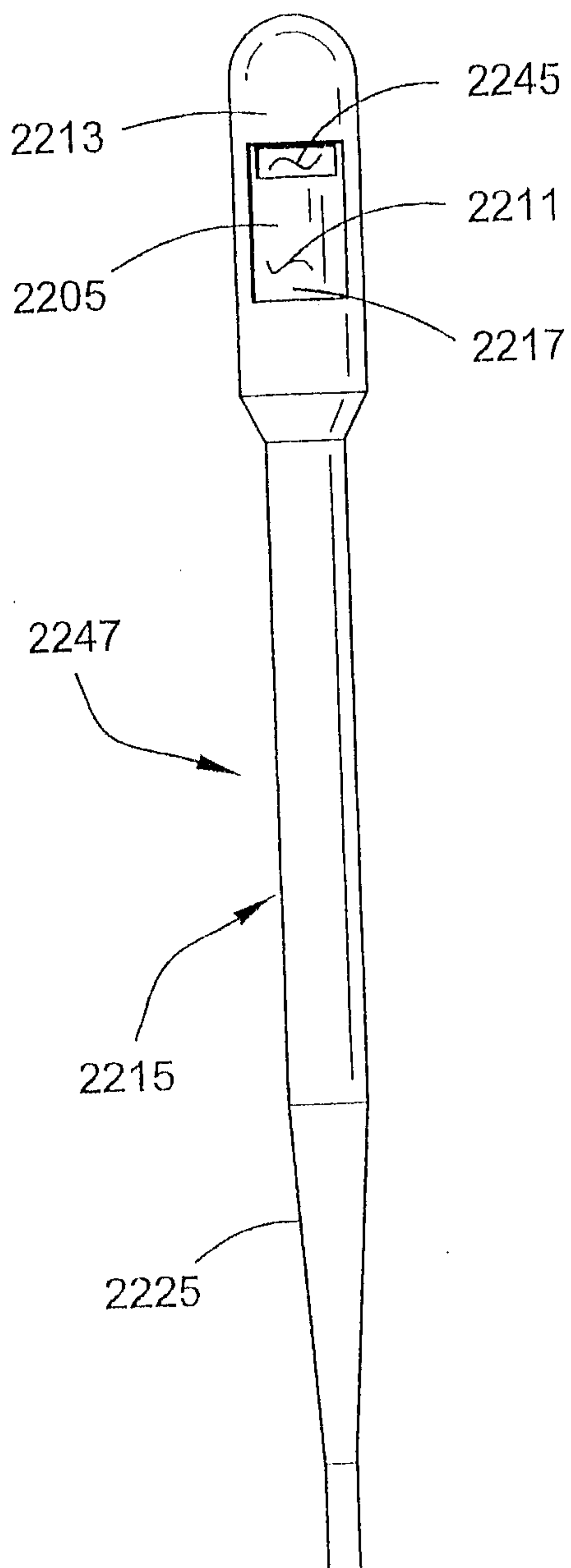
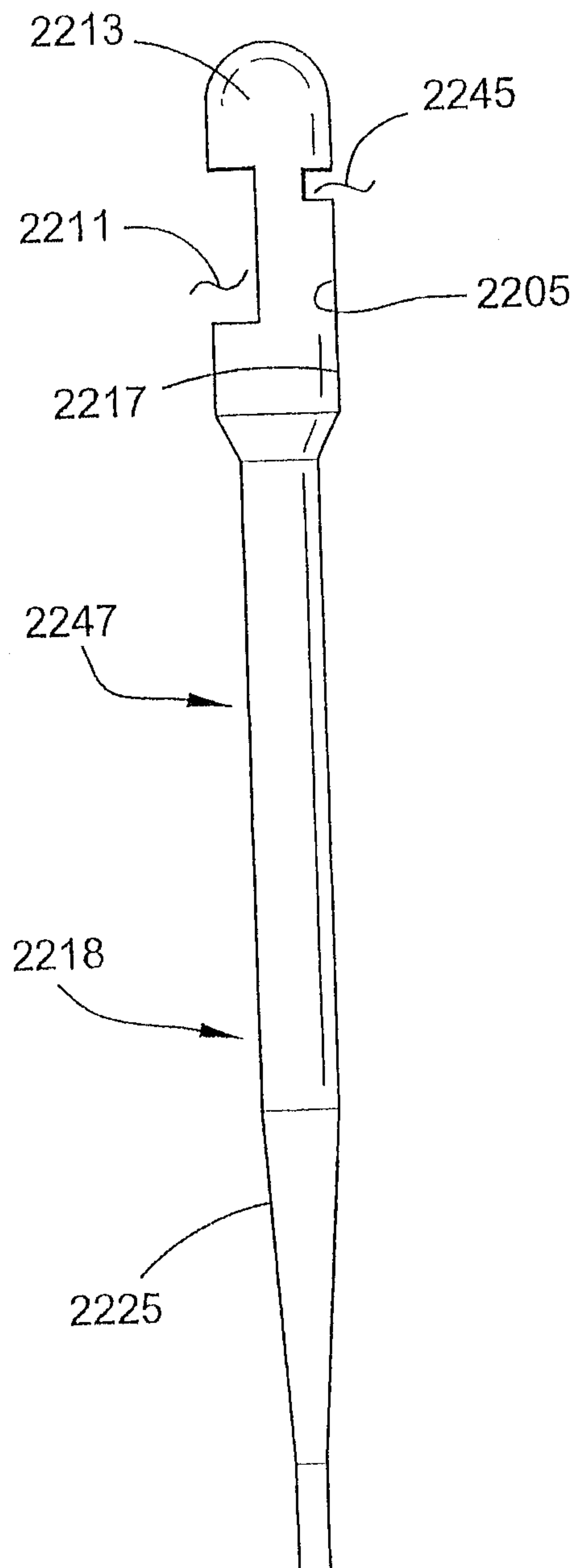


FIG. 87



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FIG. 88

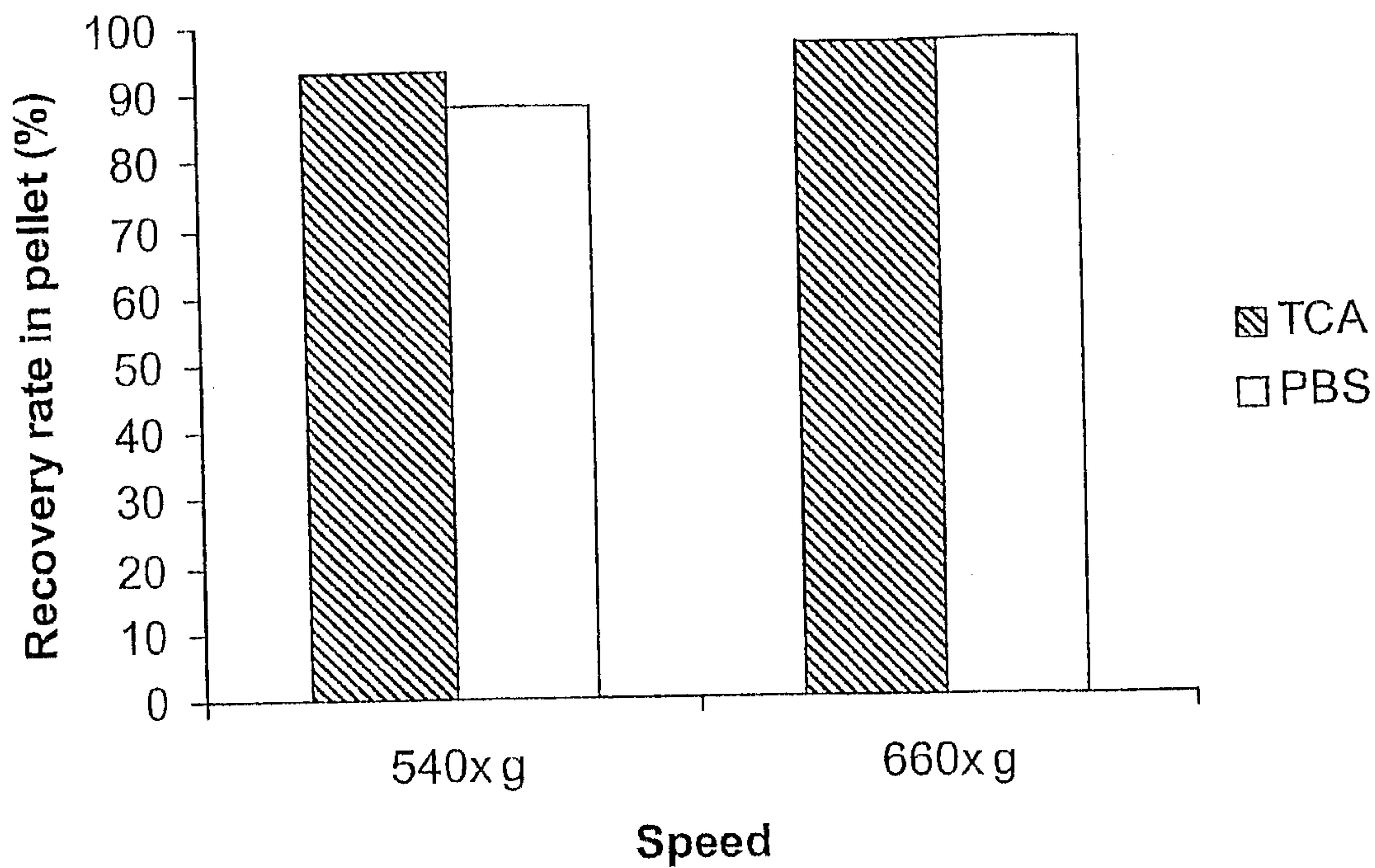
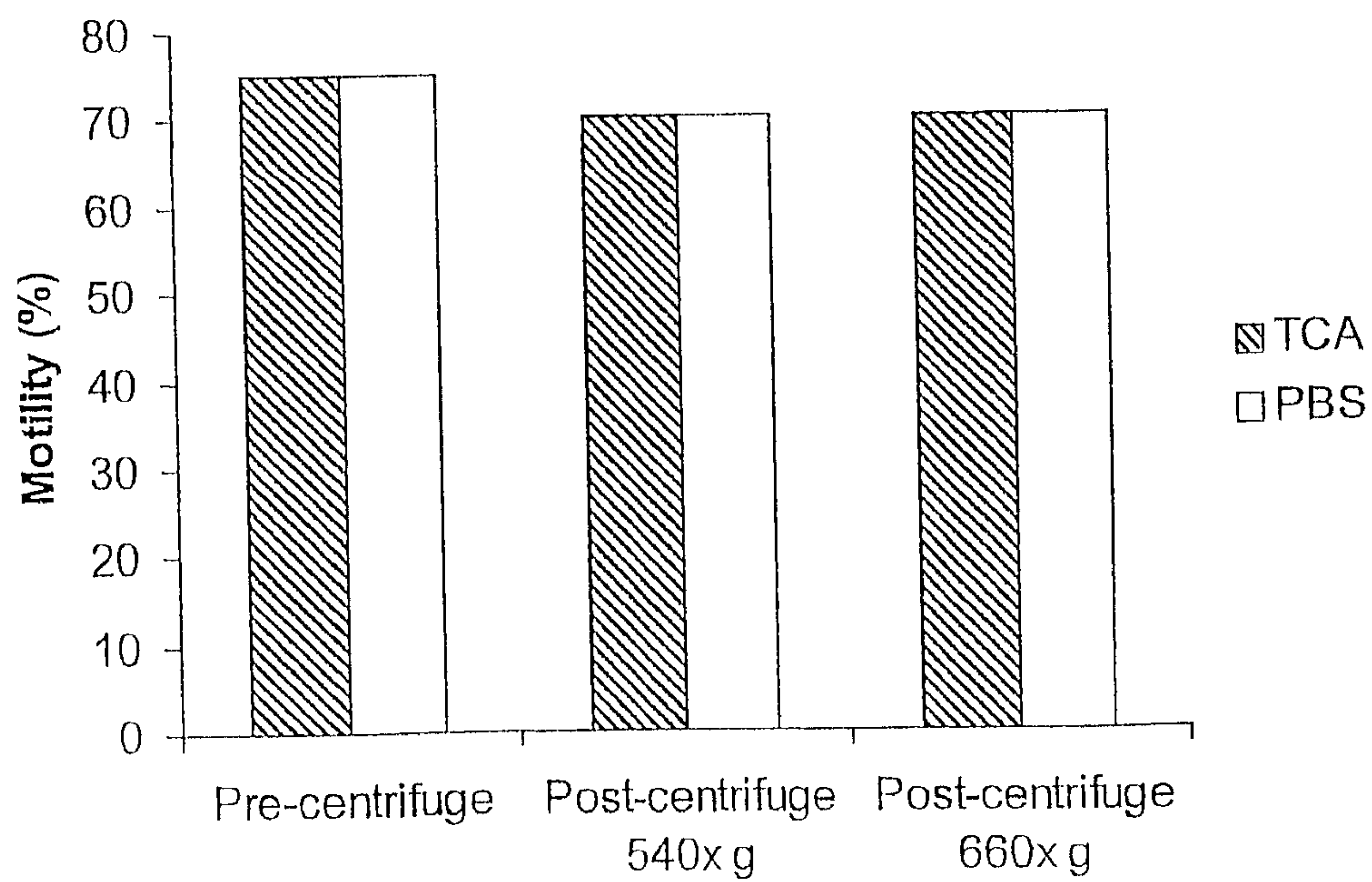


FIG. 89



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FIG. 90

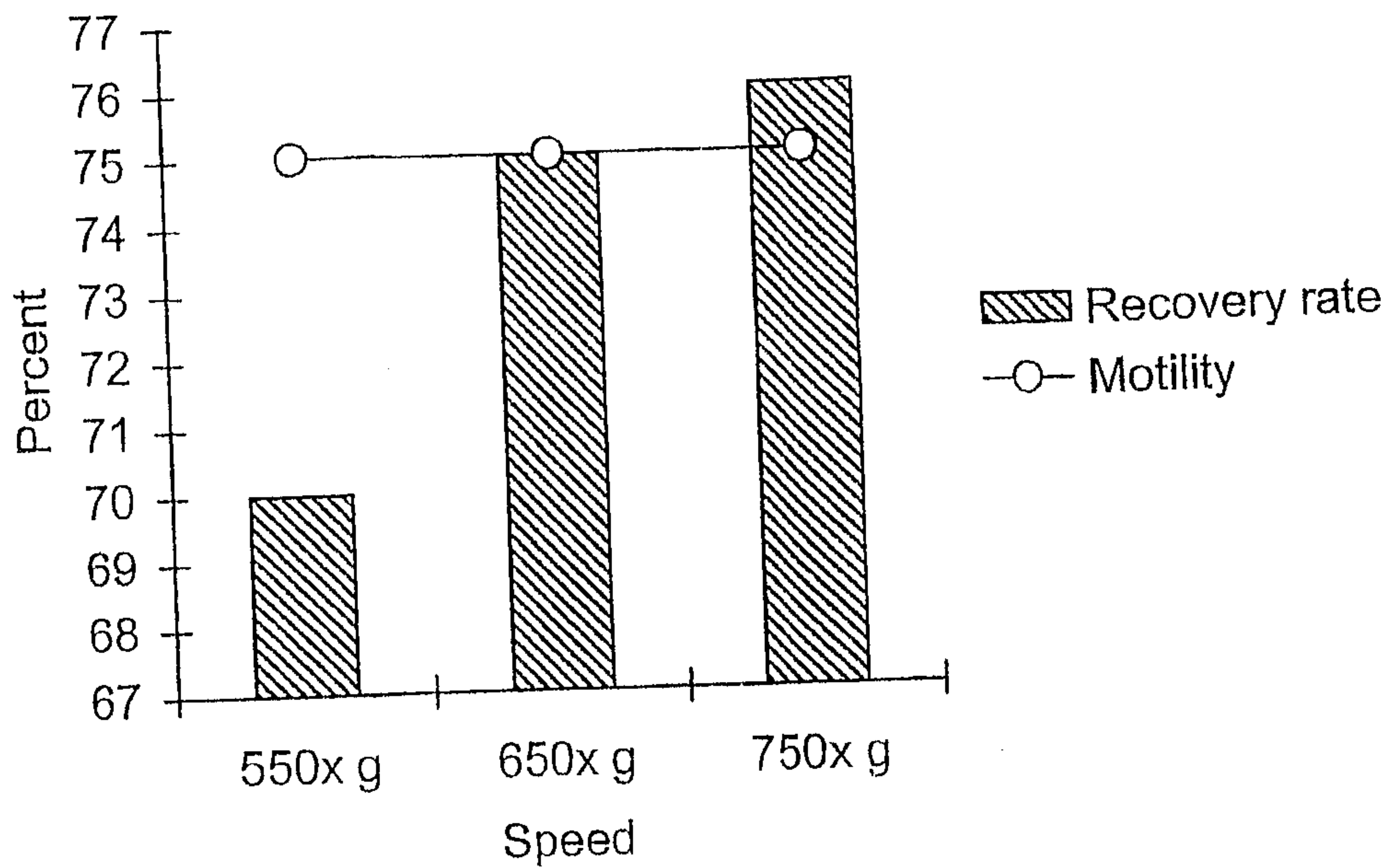
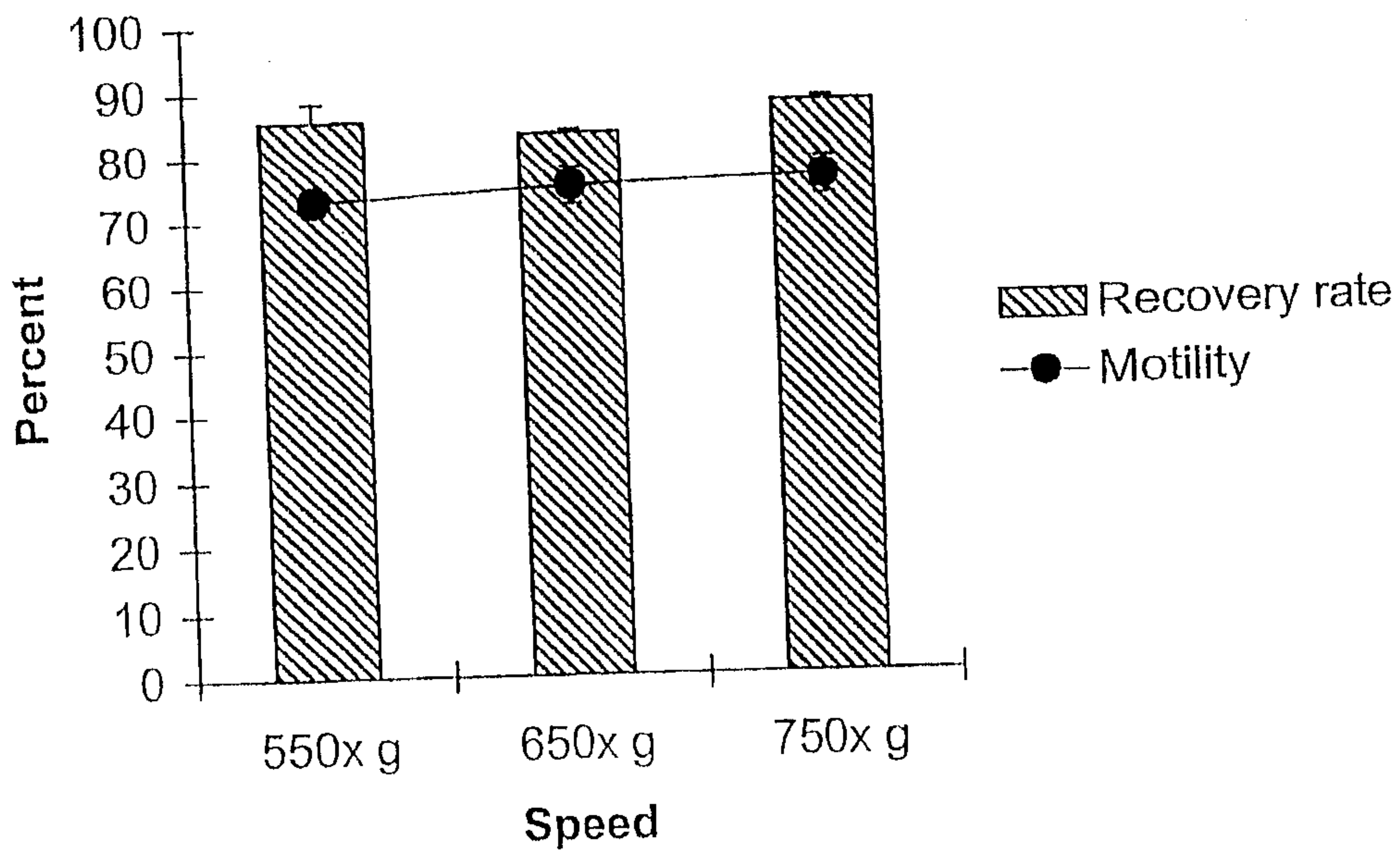


FIG. 91



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FIG. 92

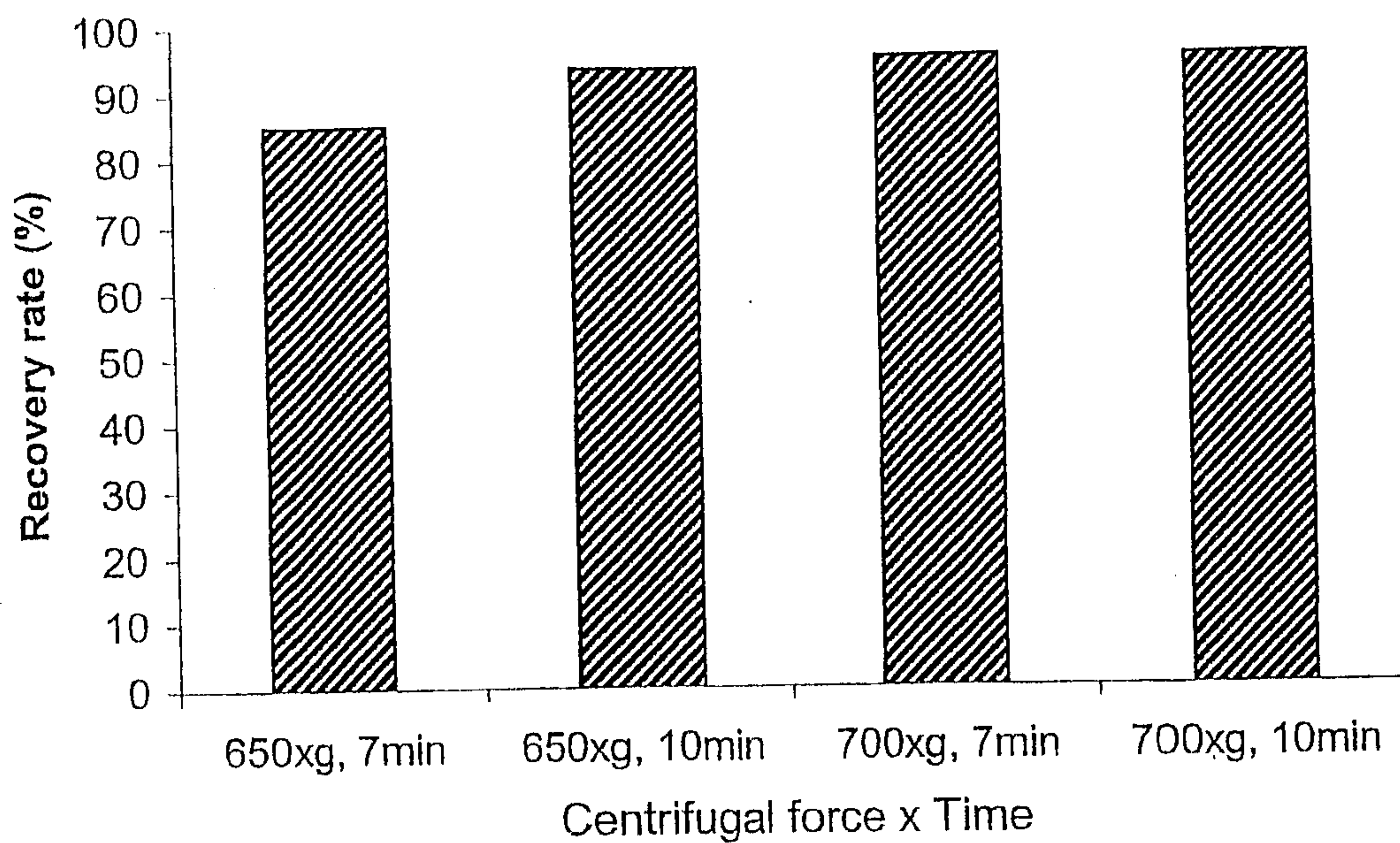
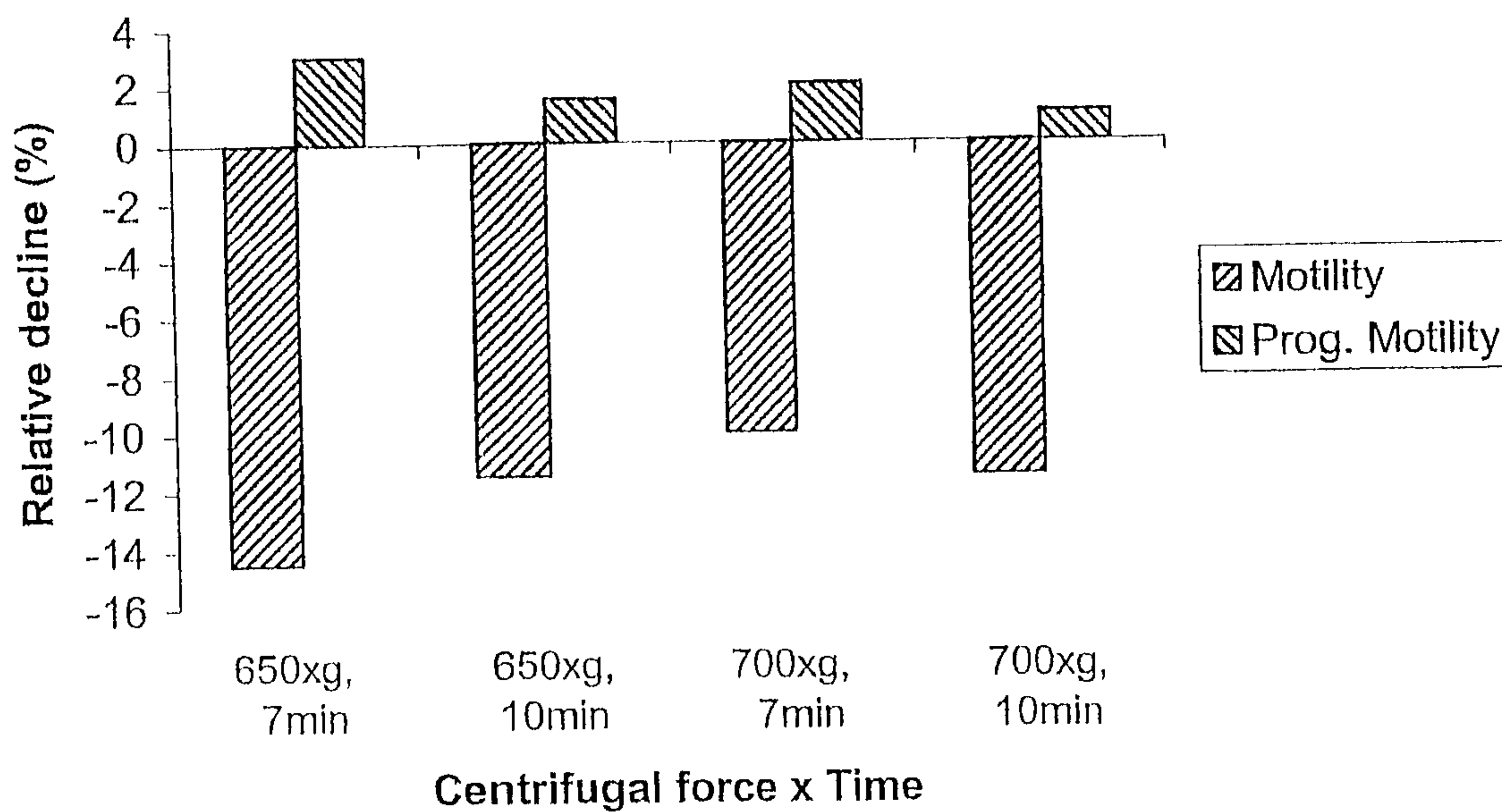


FIG. 93



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FIG. 94

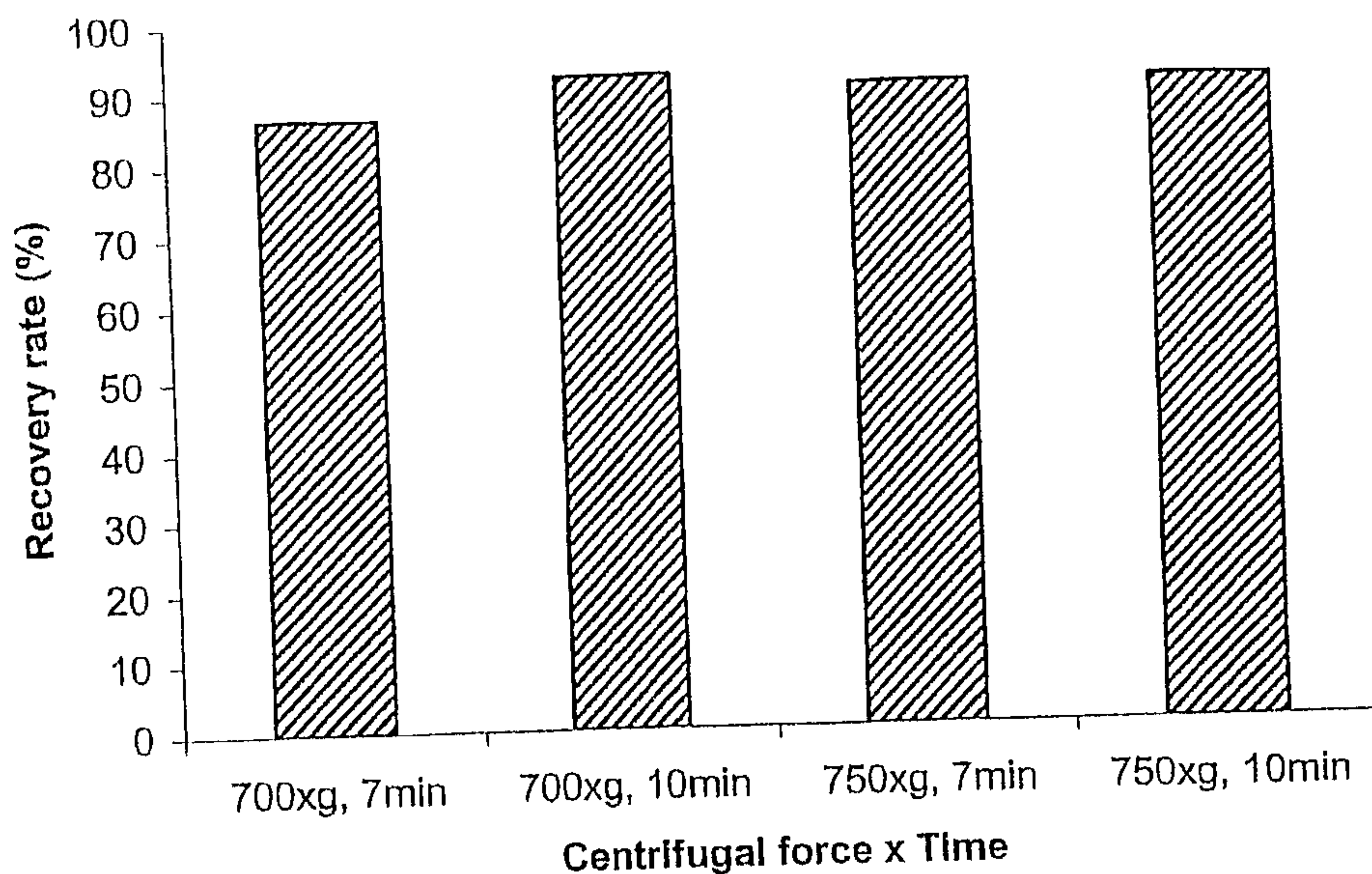
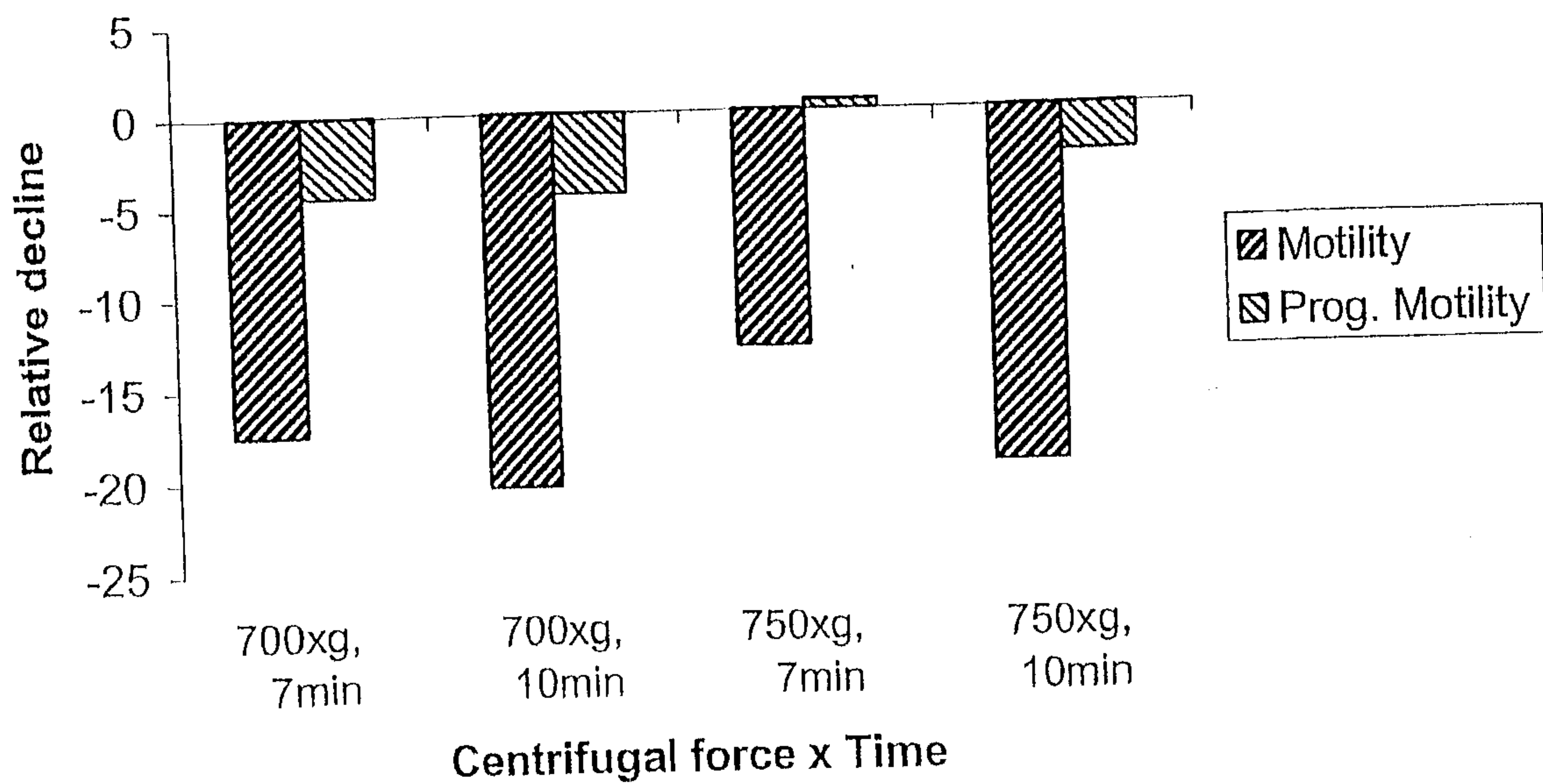


FIG. 95



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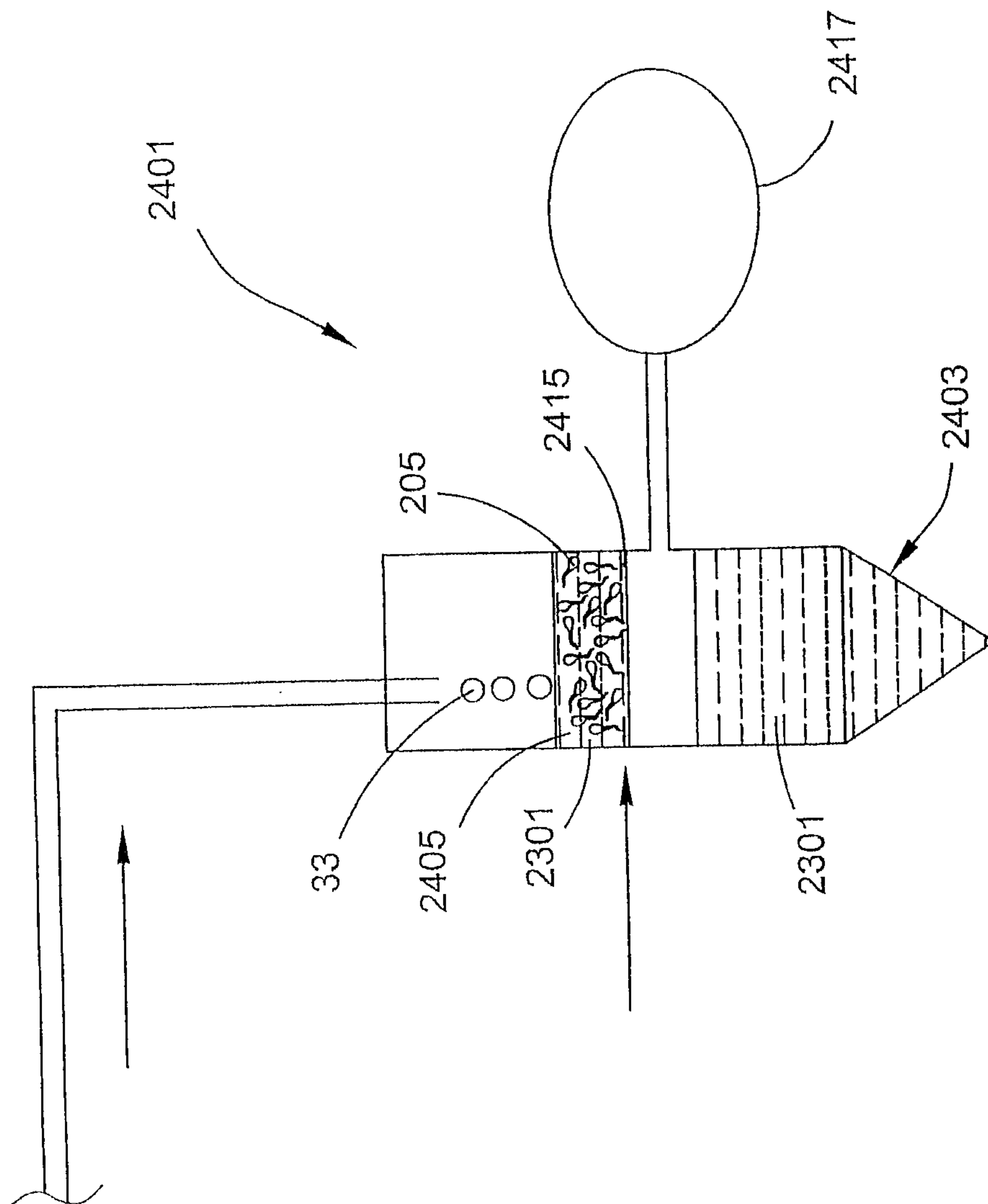


FIG. 96

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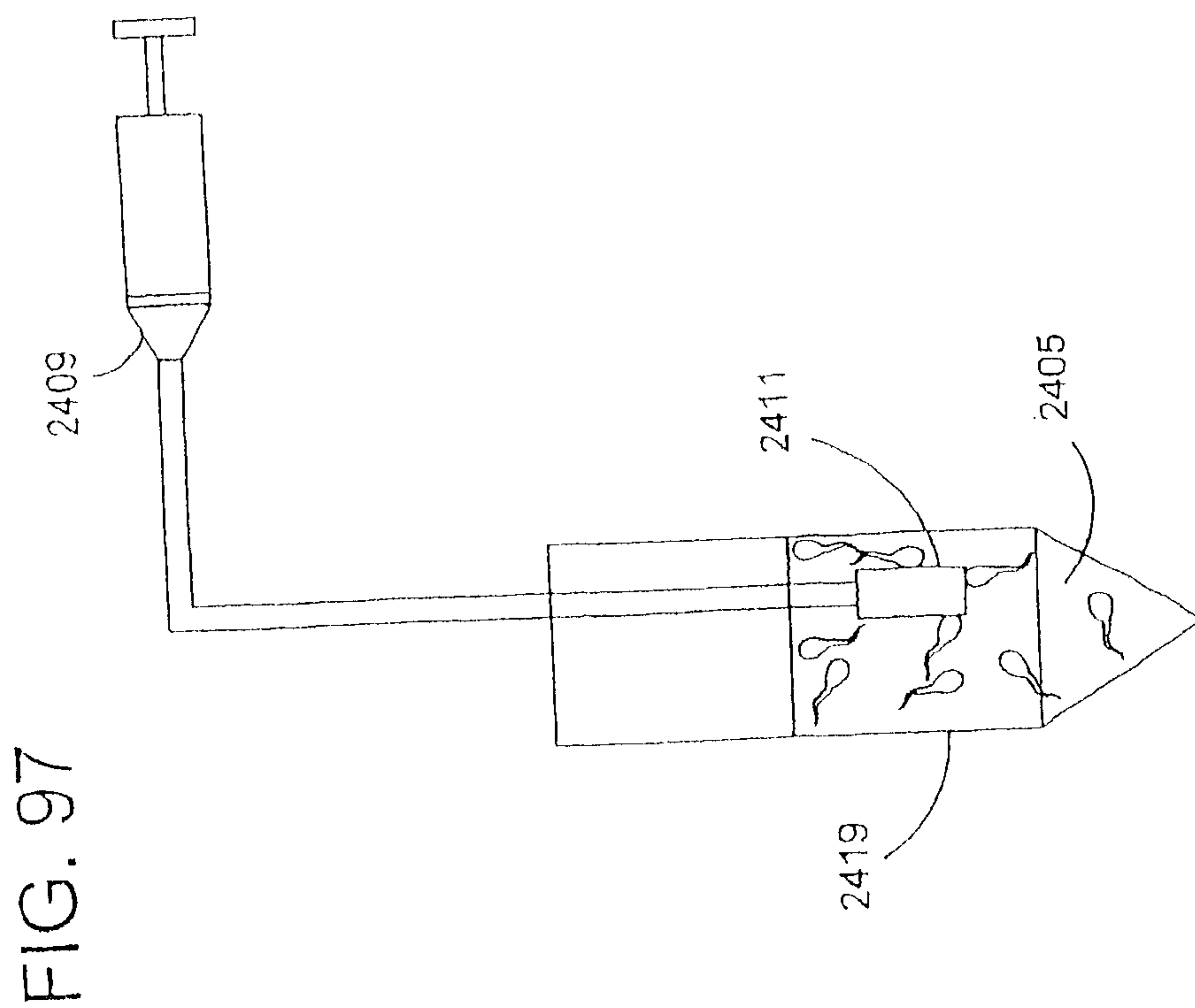
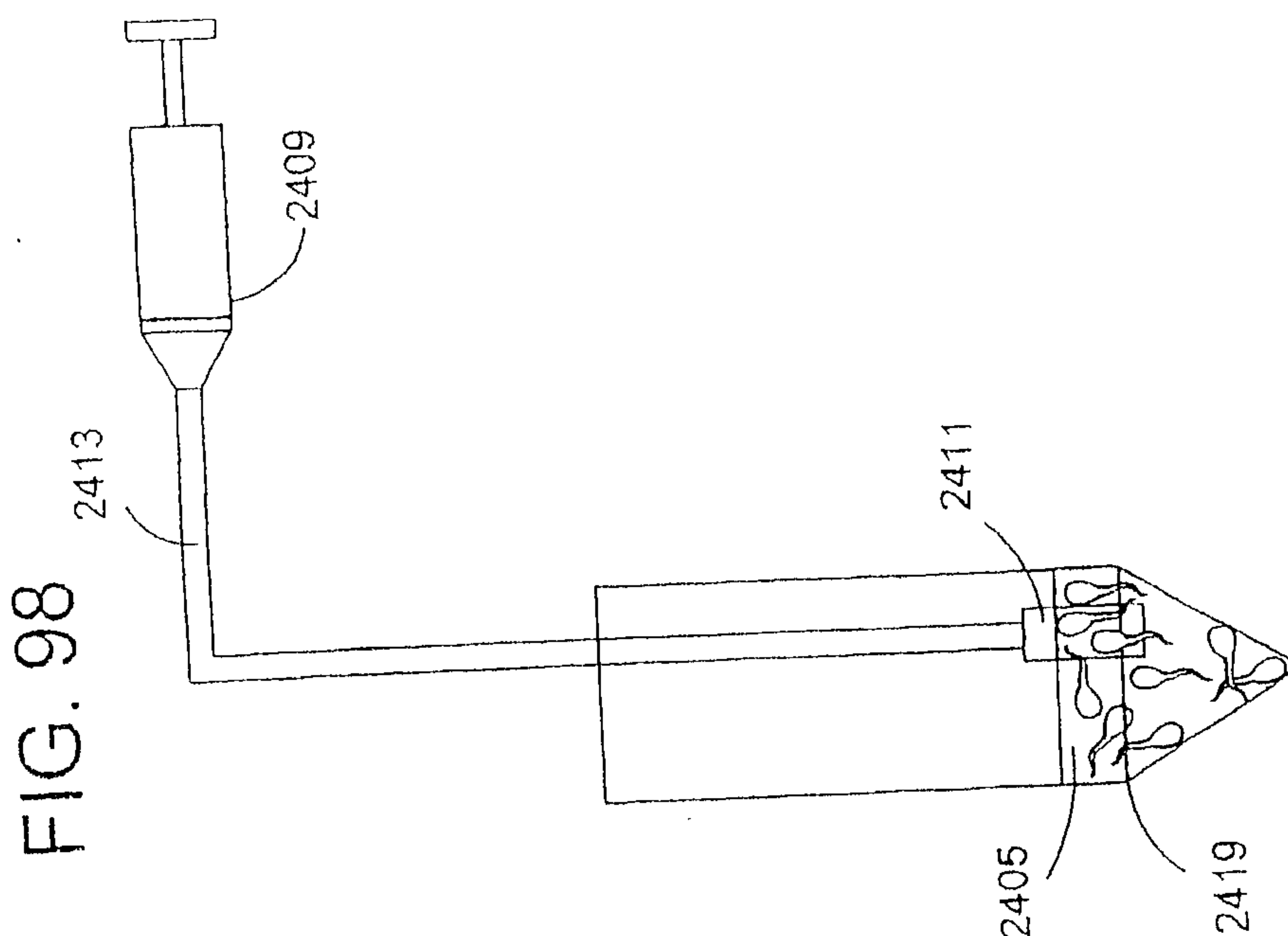


FIG. 98

FIG. 97

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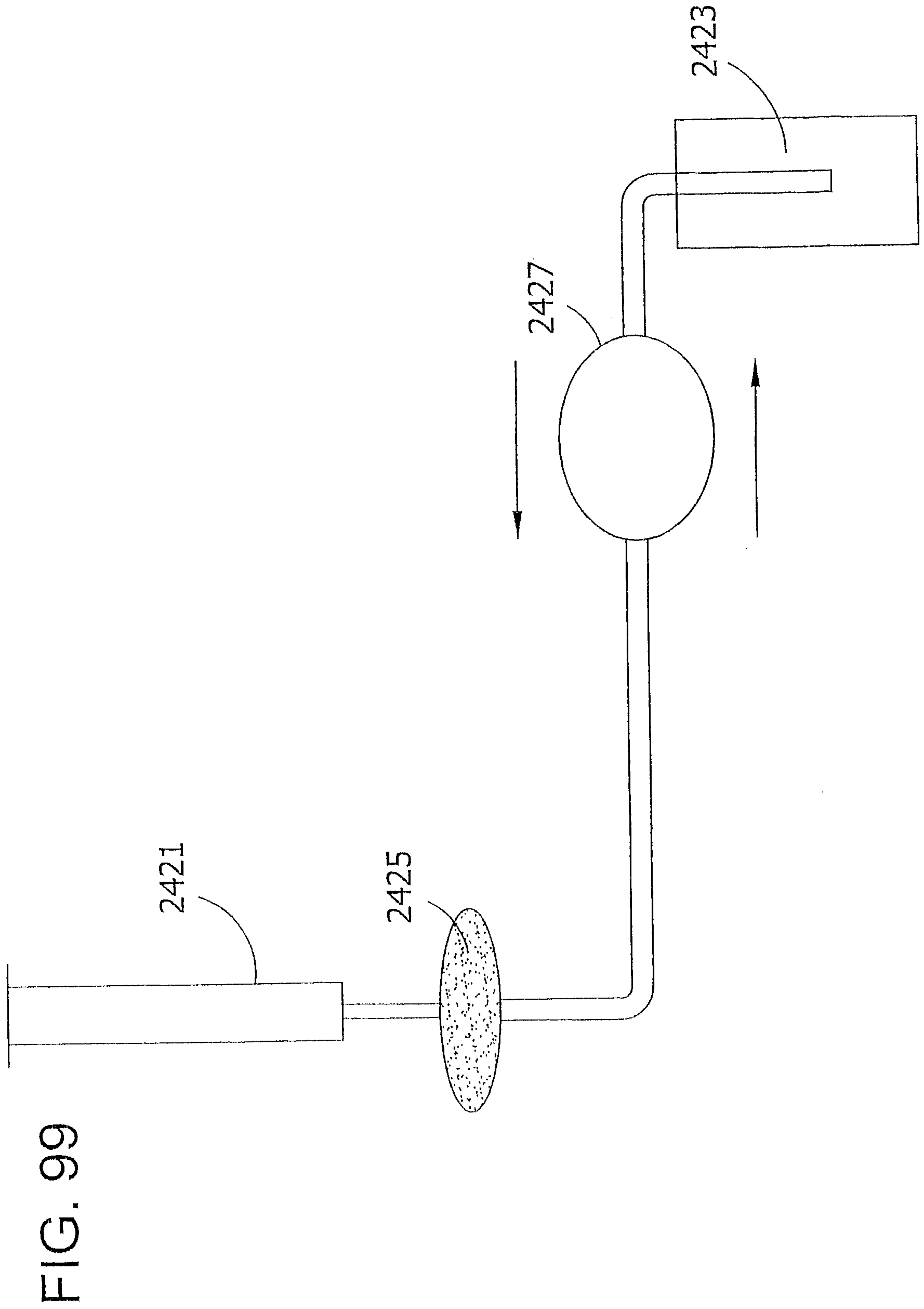


FIG. 99

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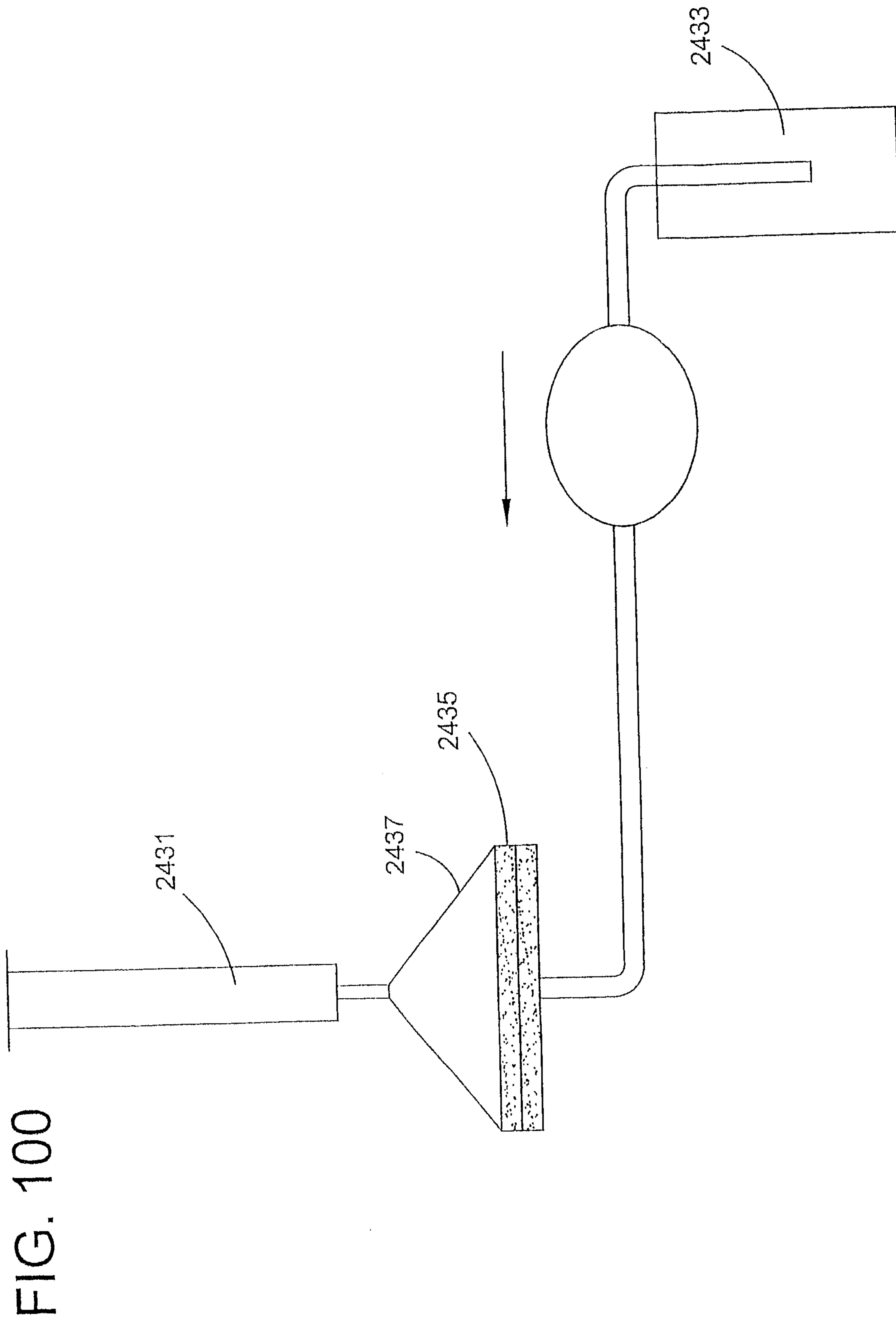


FIG. 100

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FIG. 101

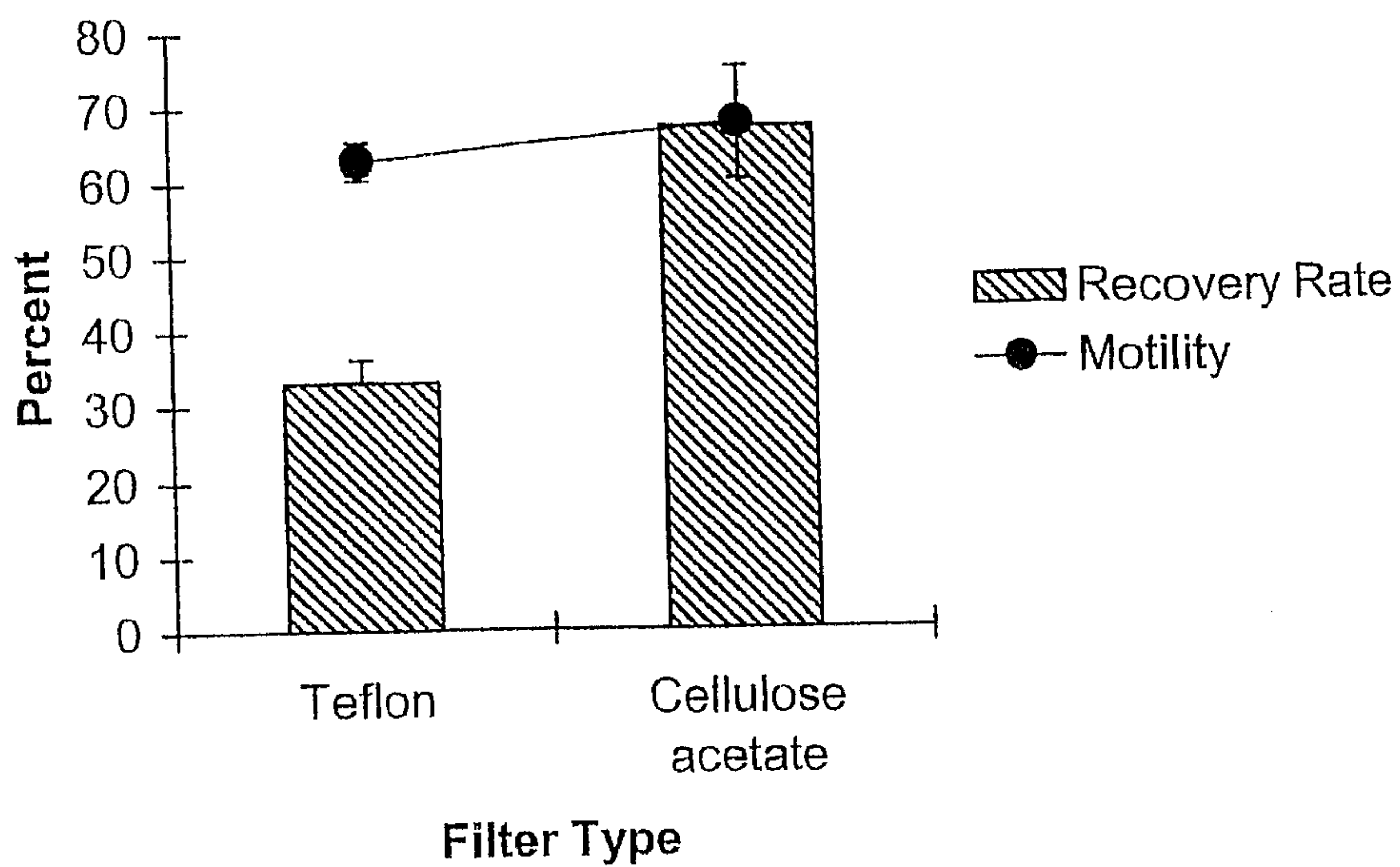
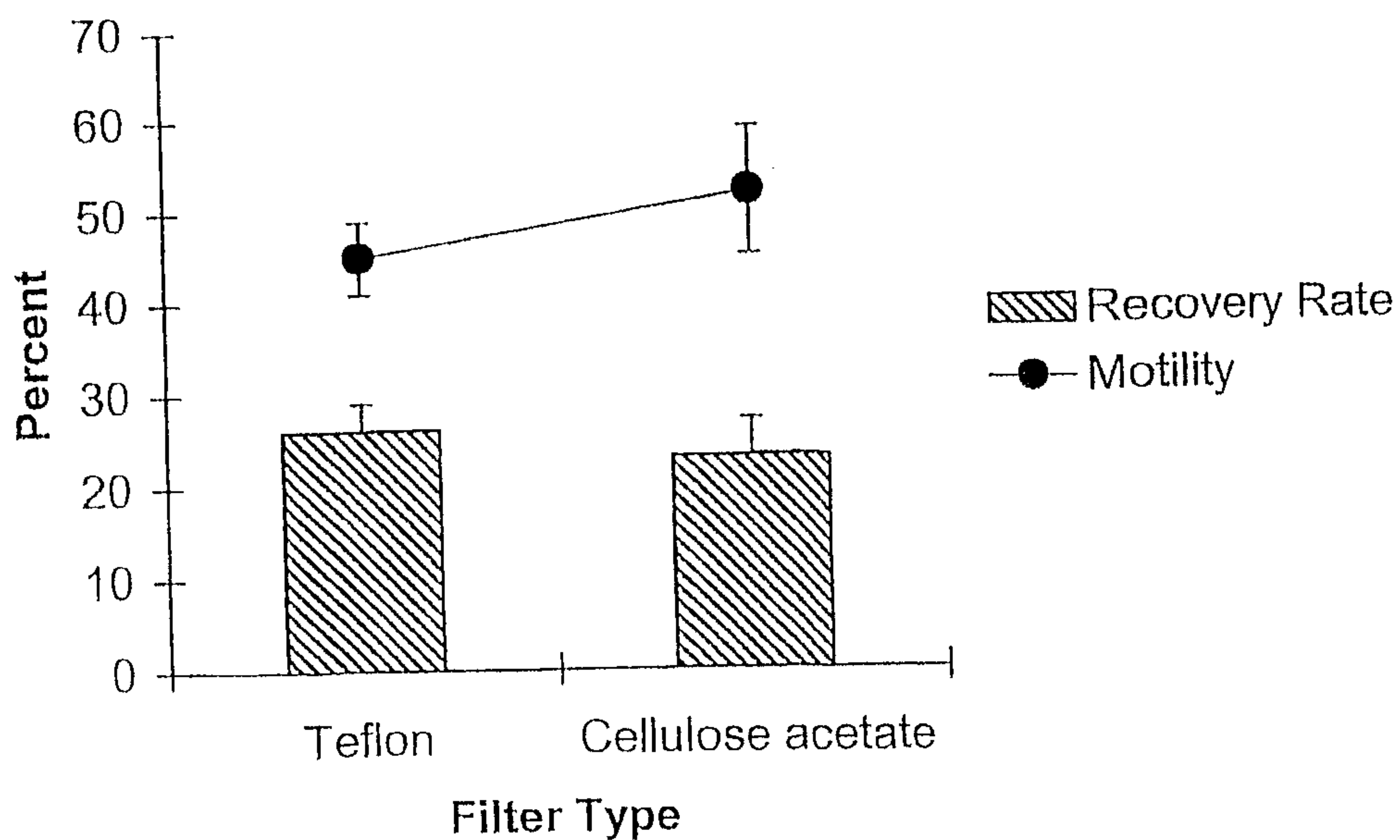
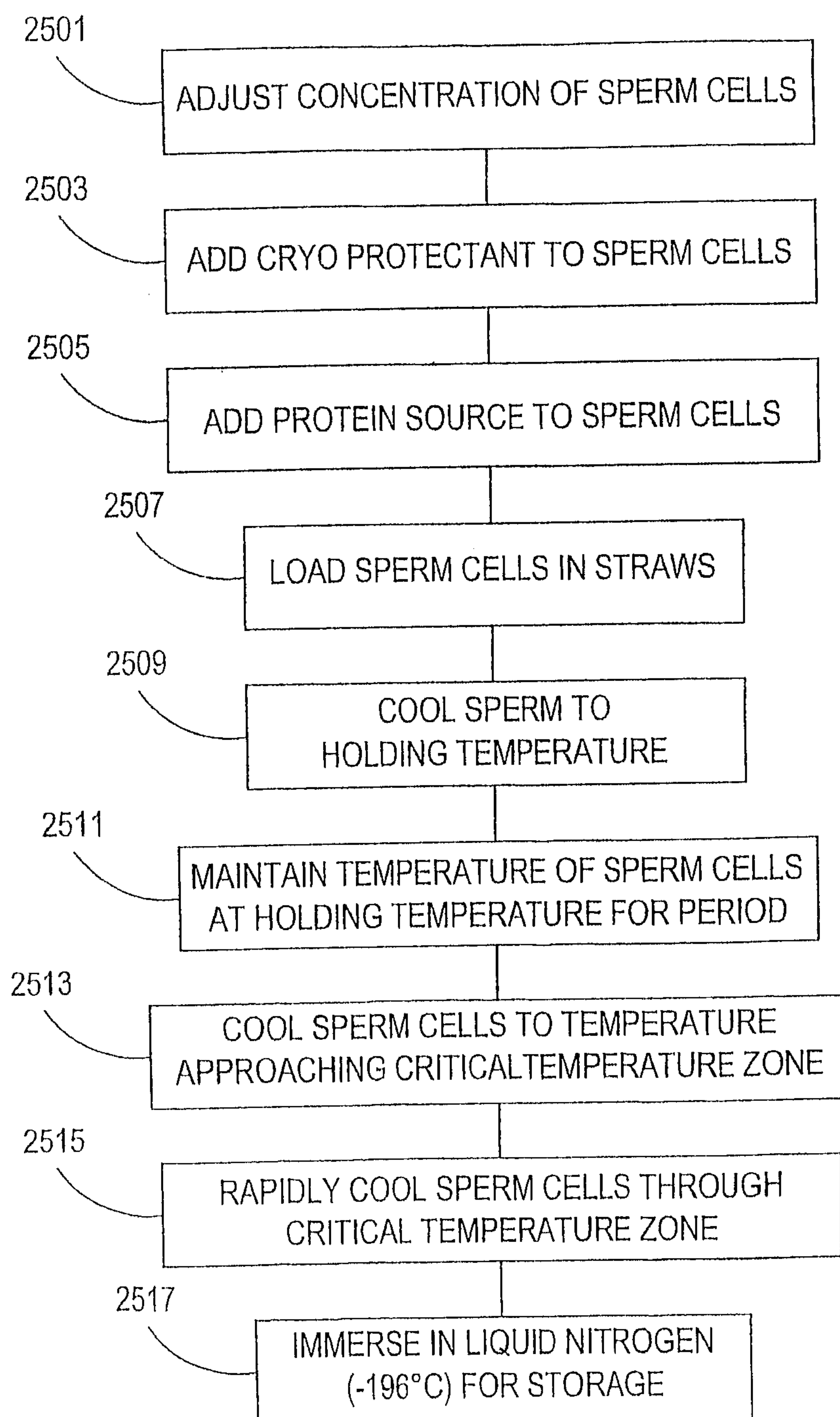


FIG. 102



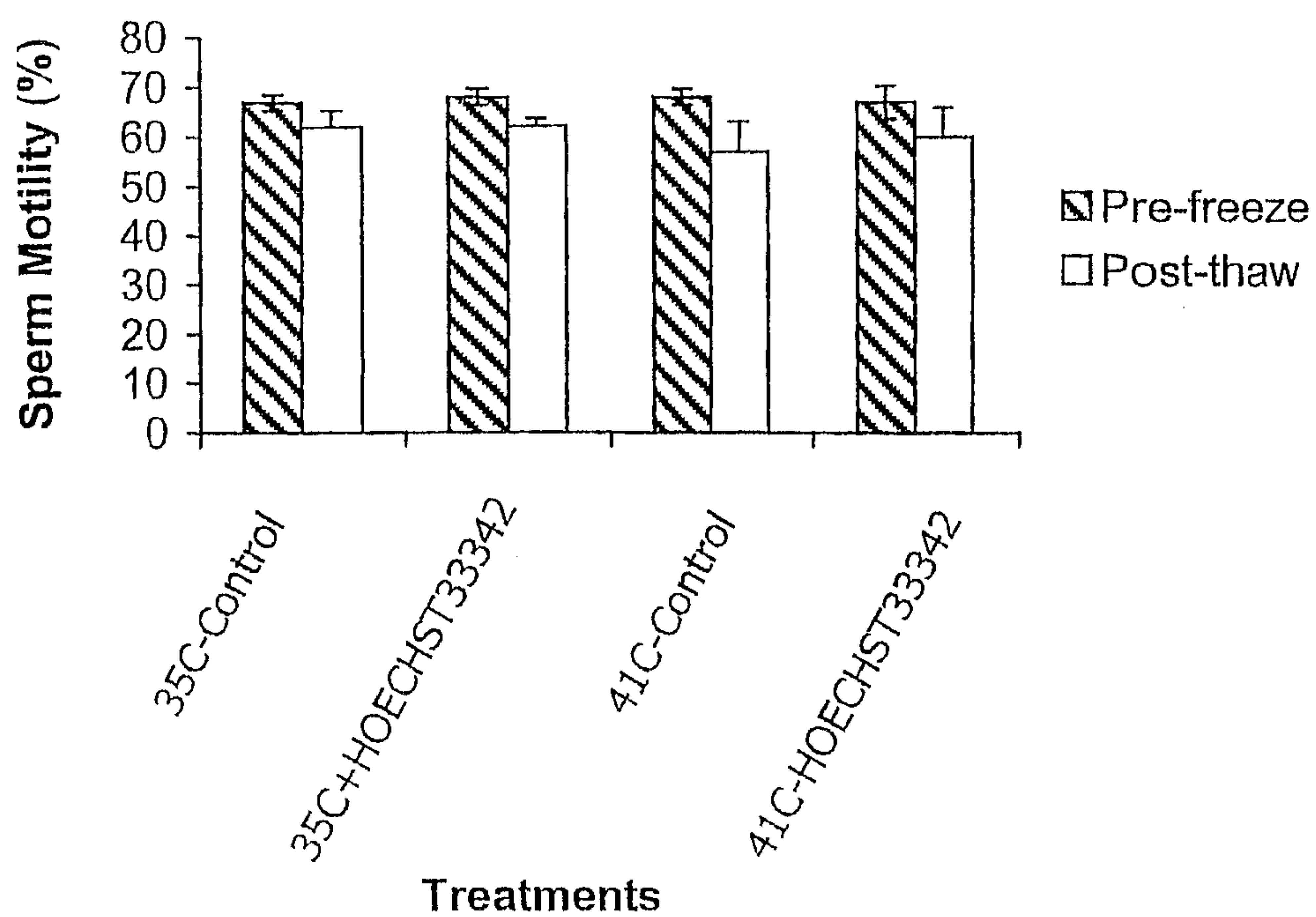
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FIG. 105

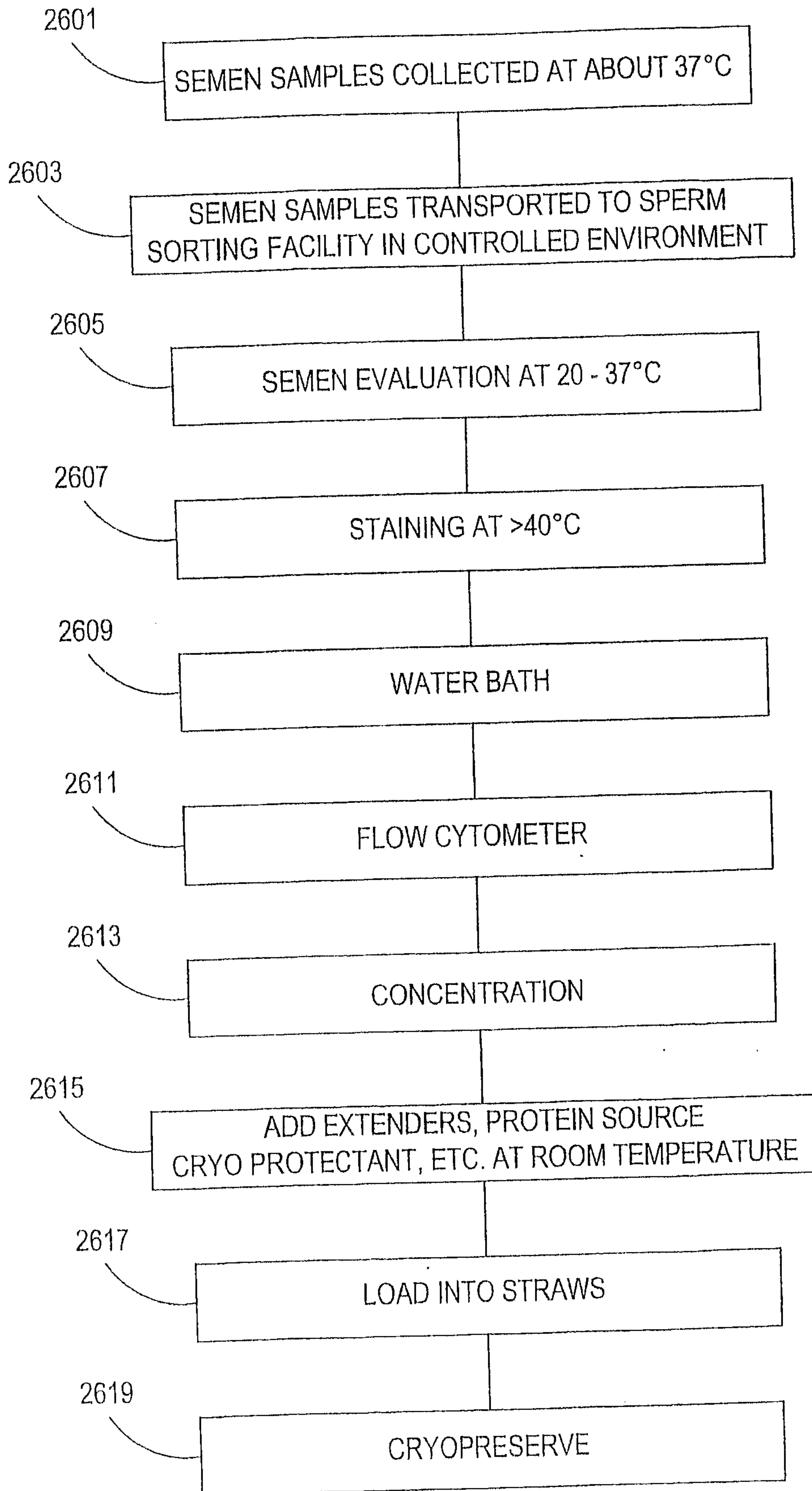


FIG. 107

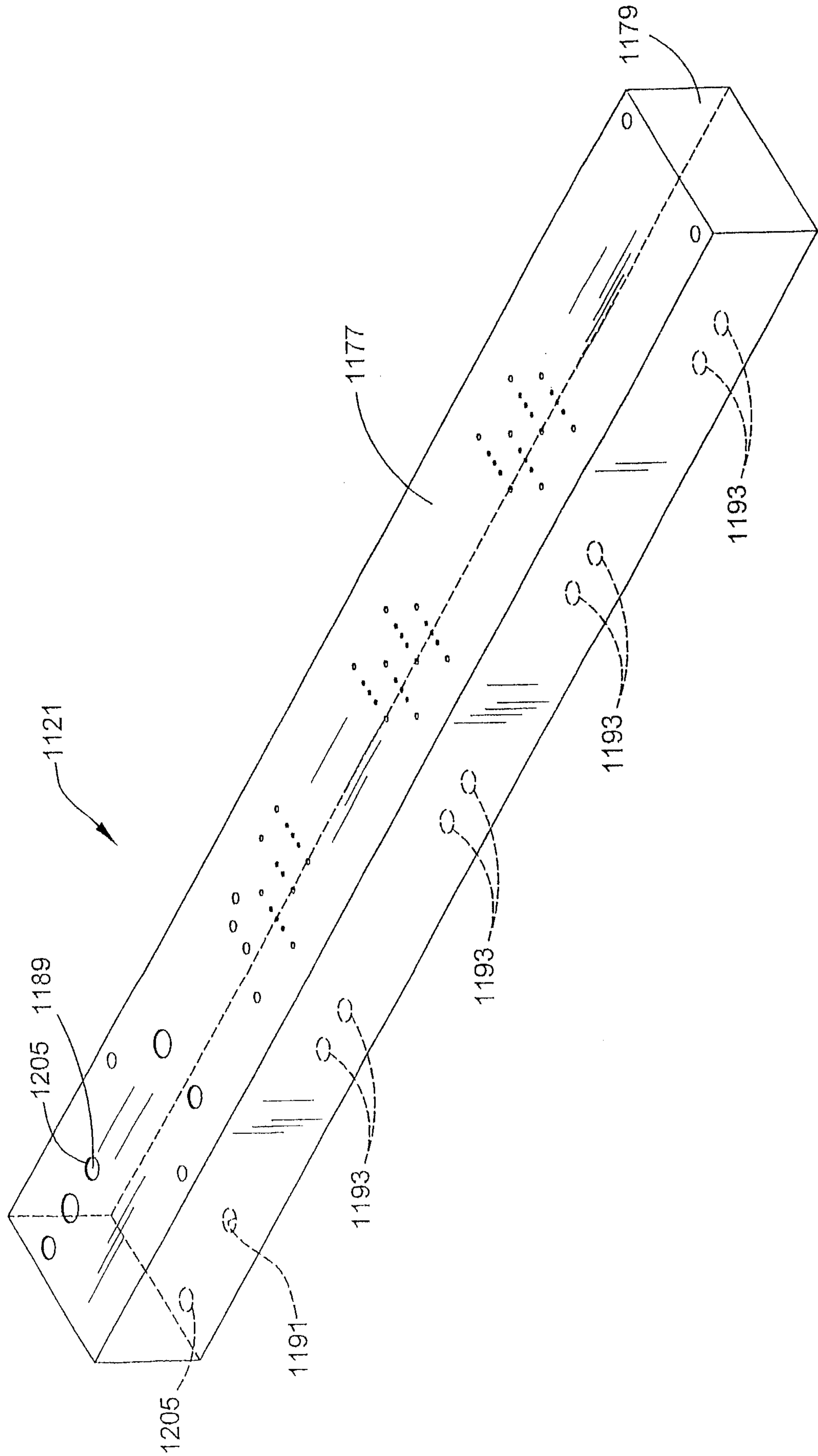
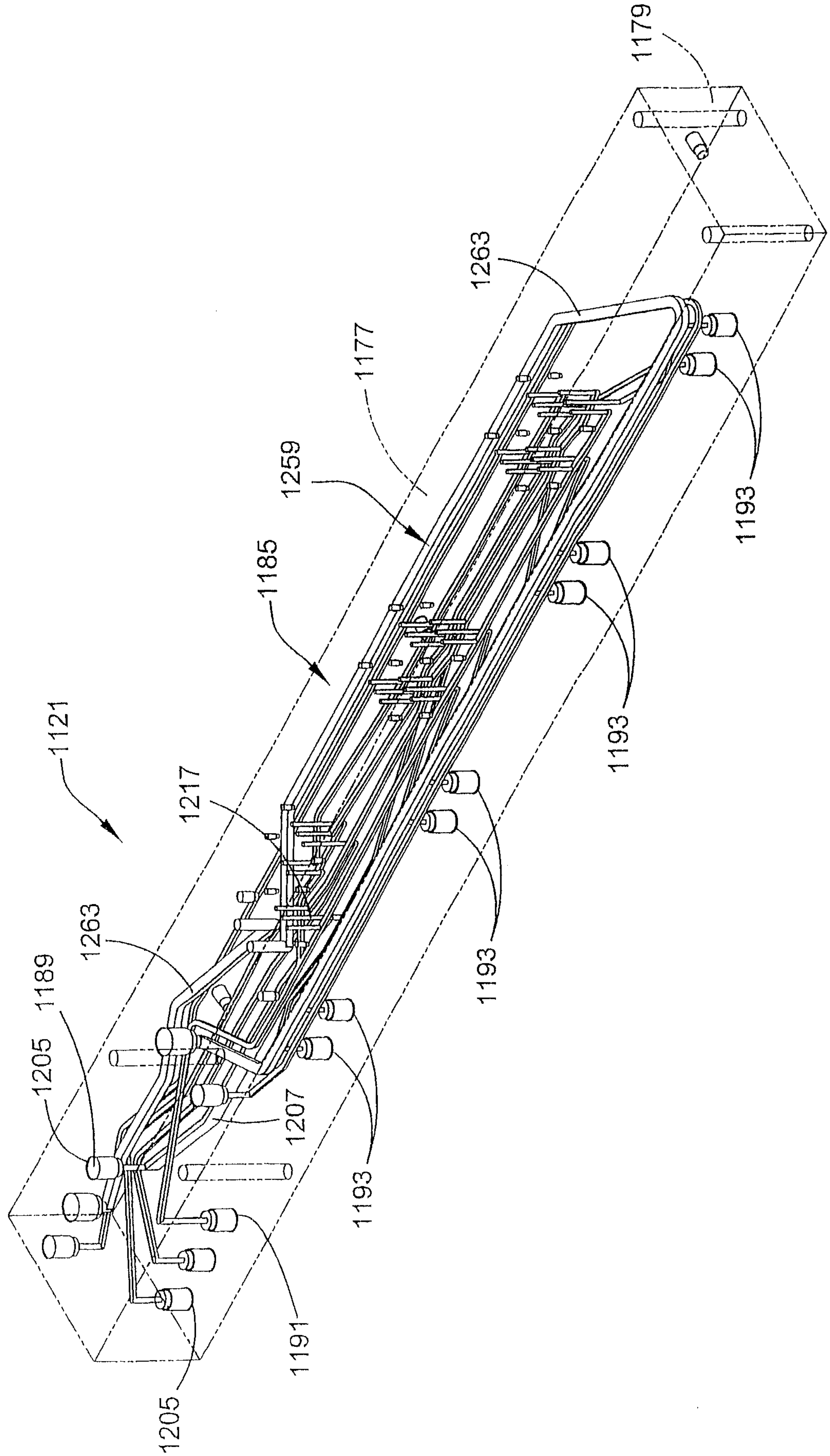


FIG. 108



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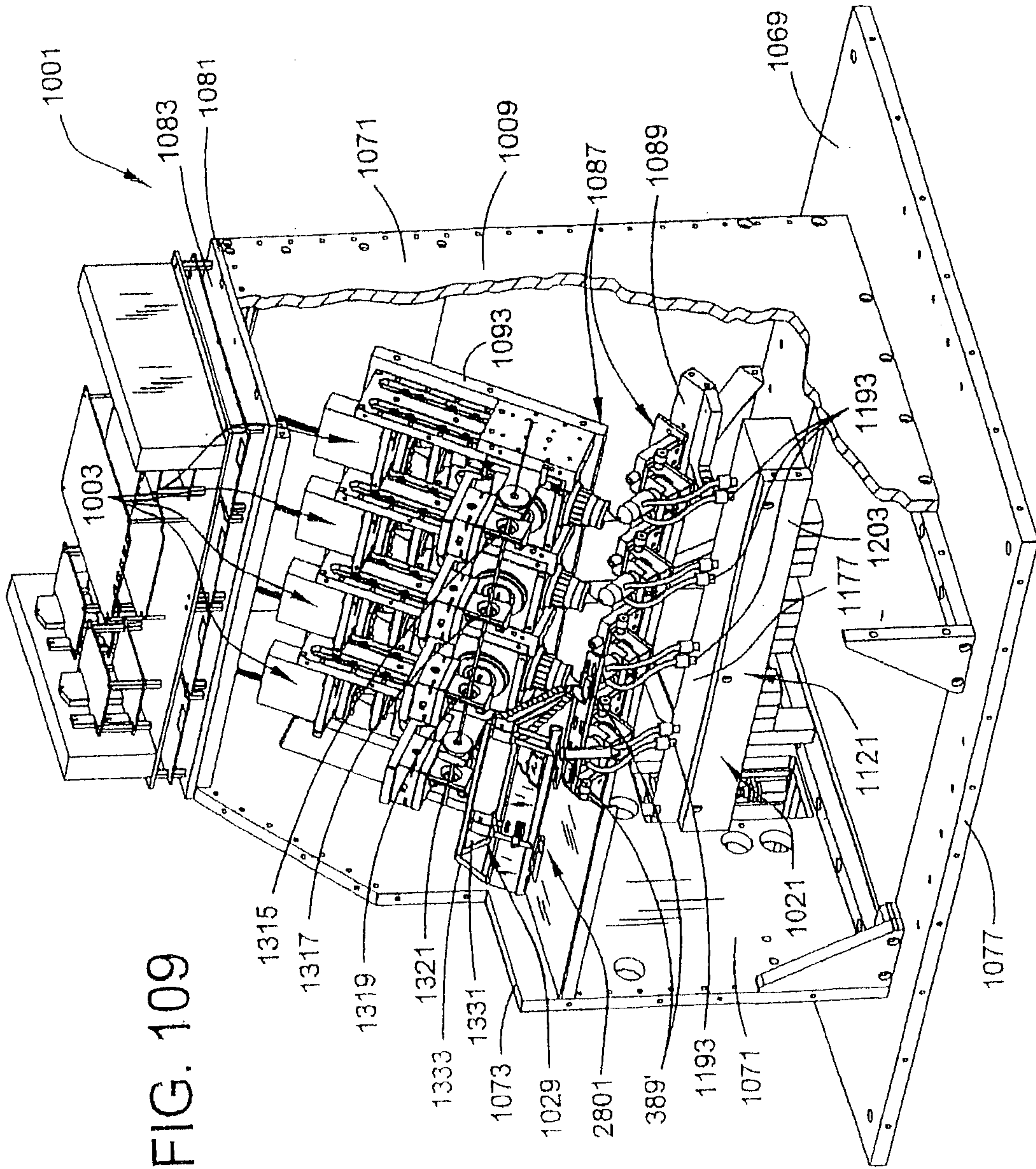
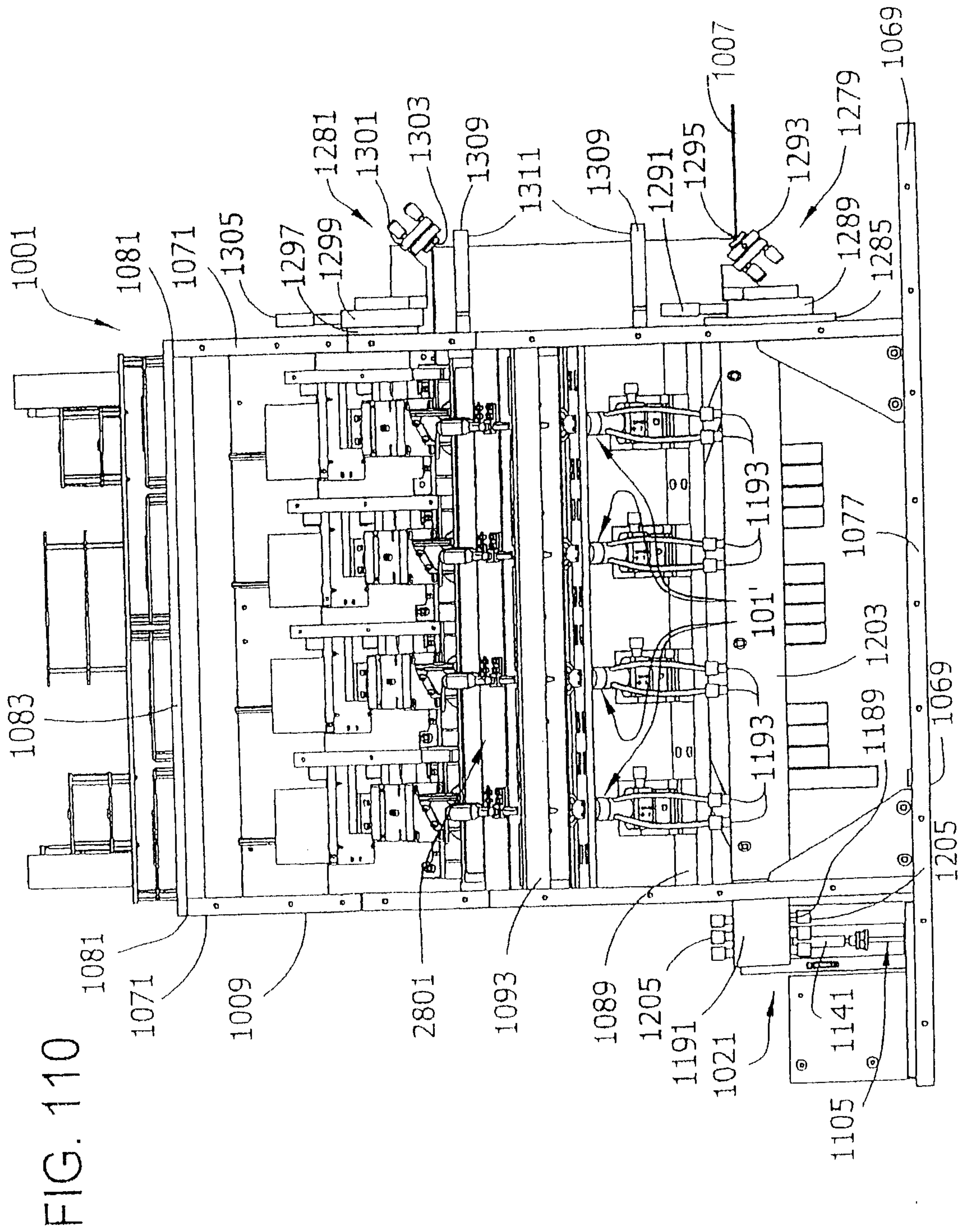


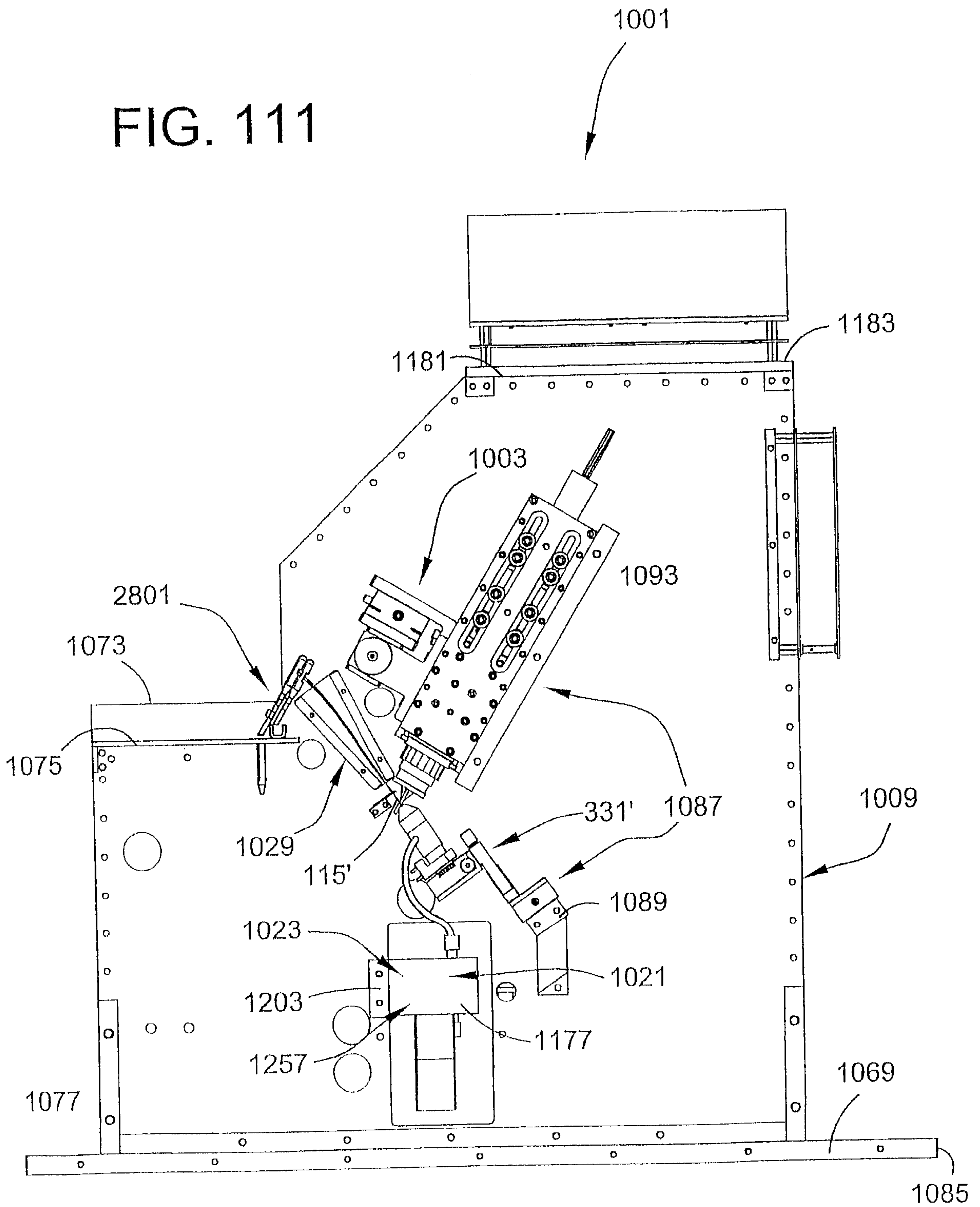
FIG. 109

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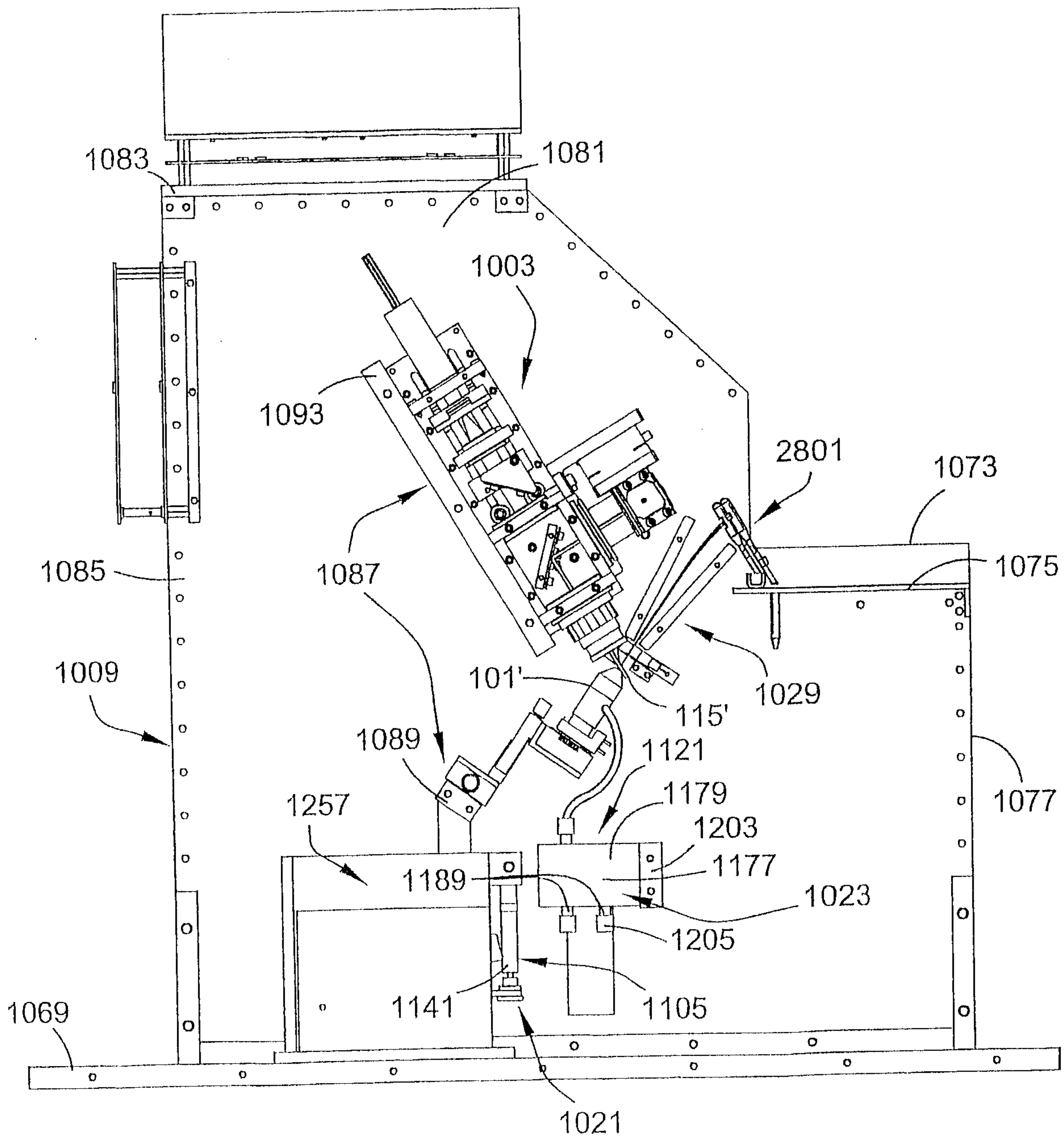
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FIG. 111



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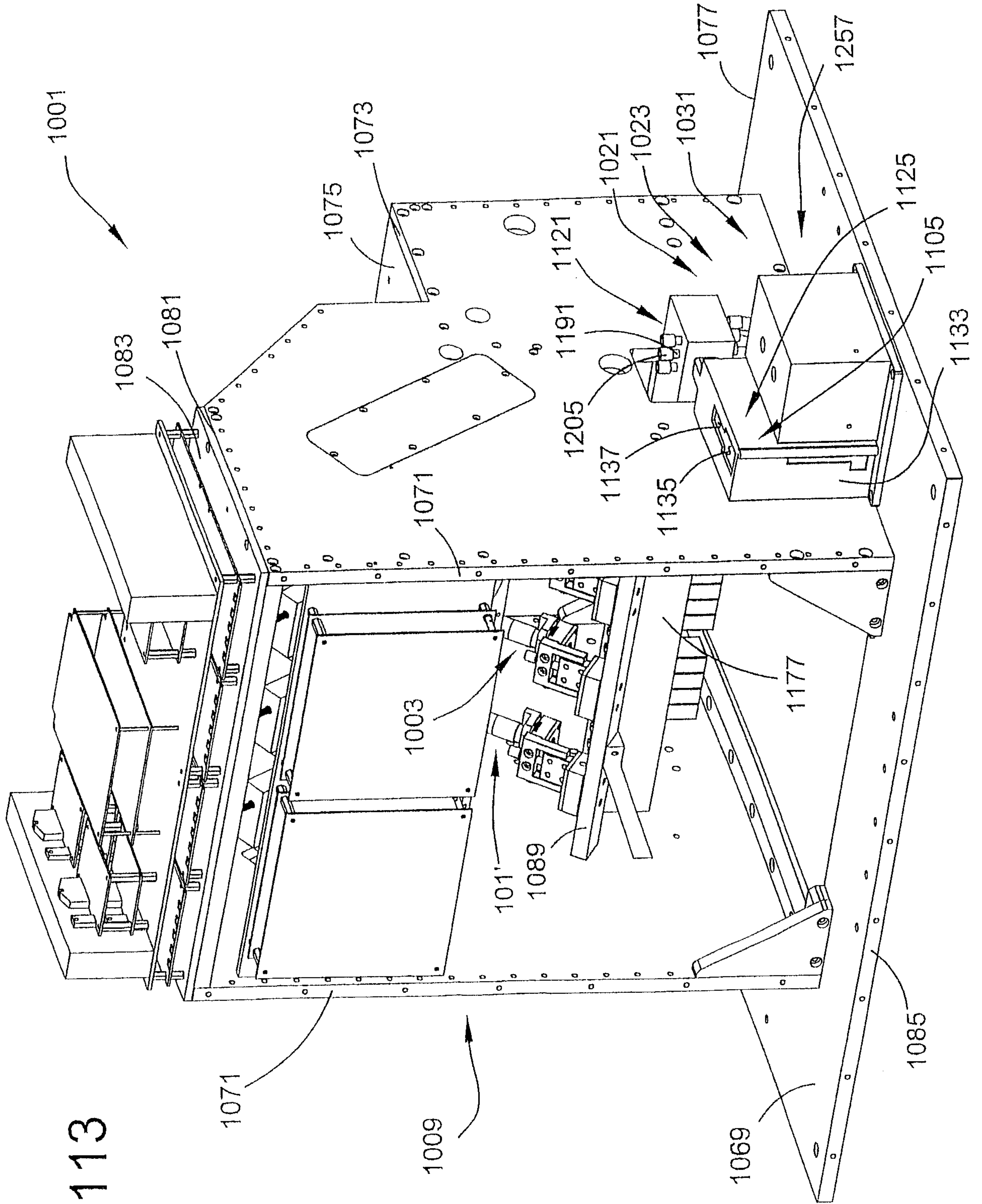


FIG. 113

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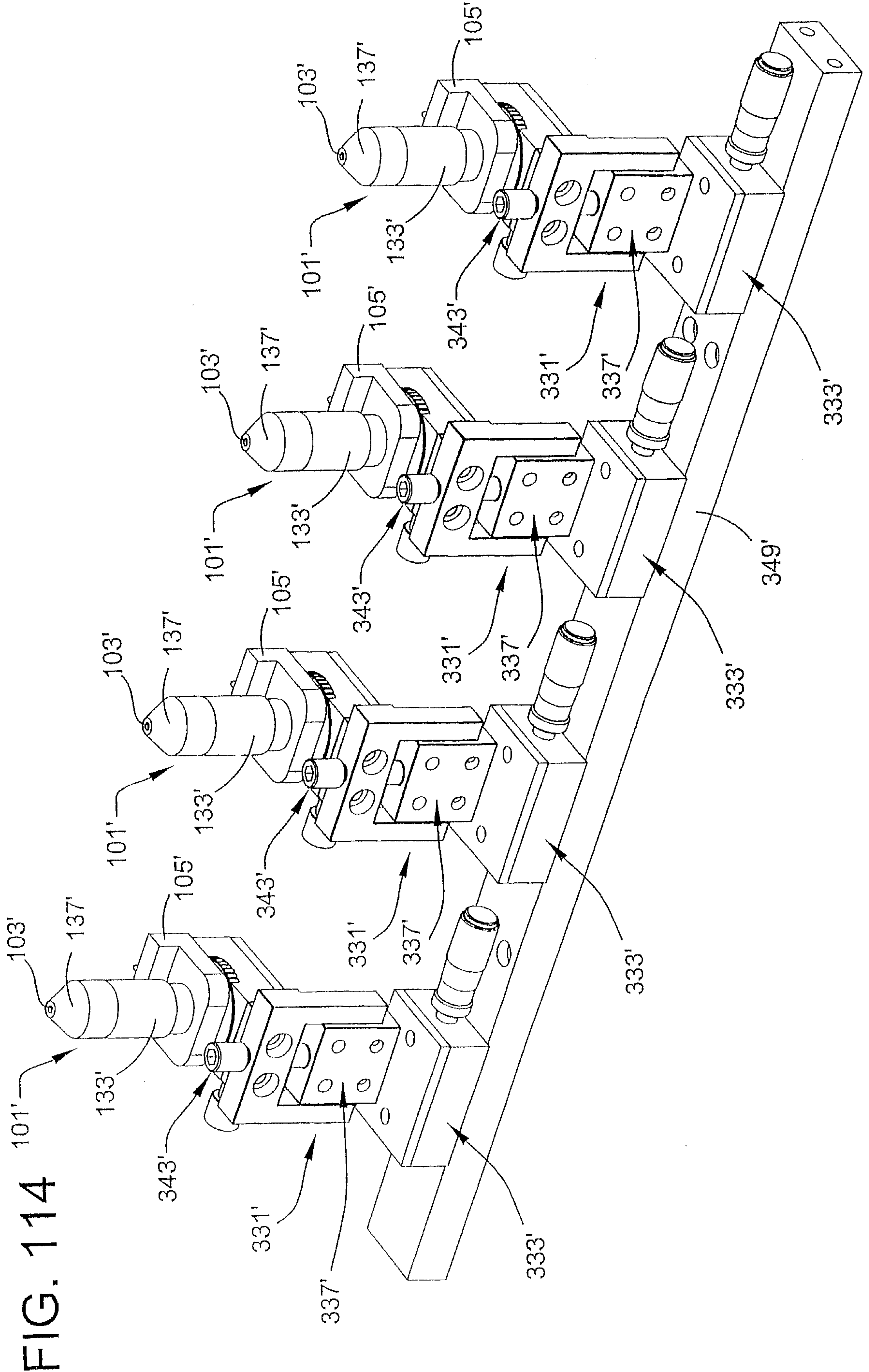


FIG. 114

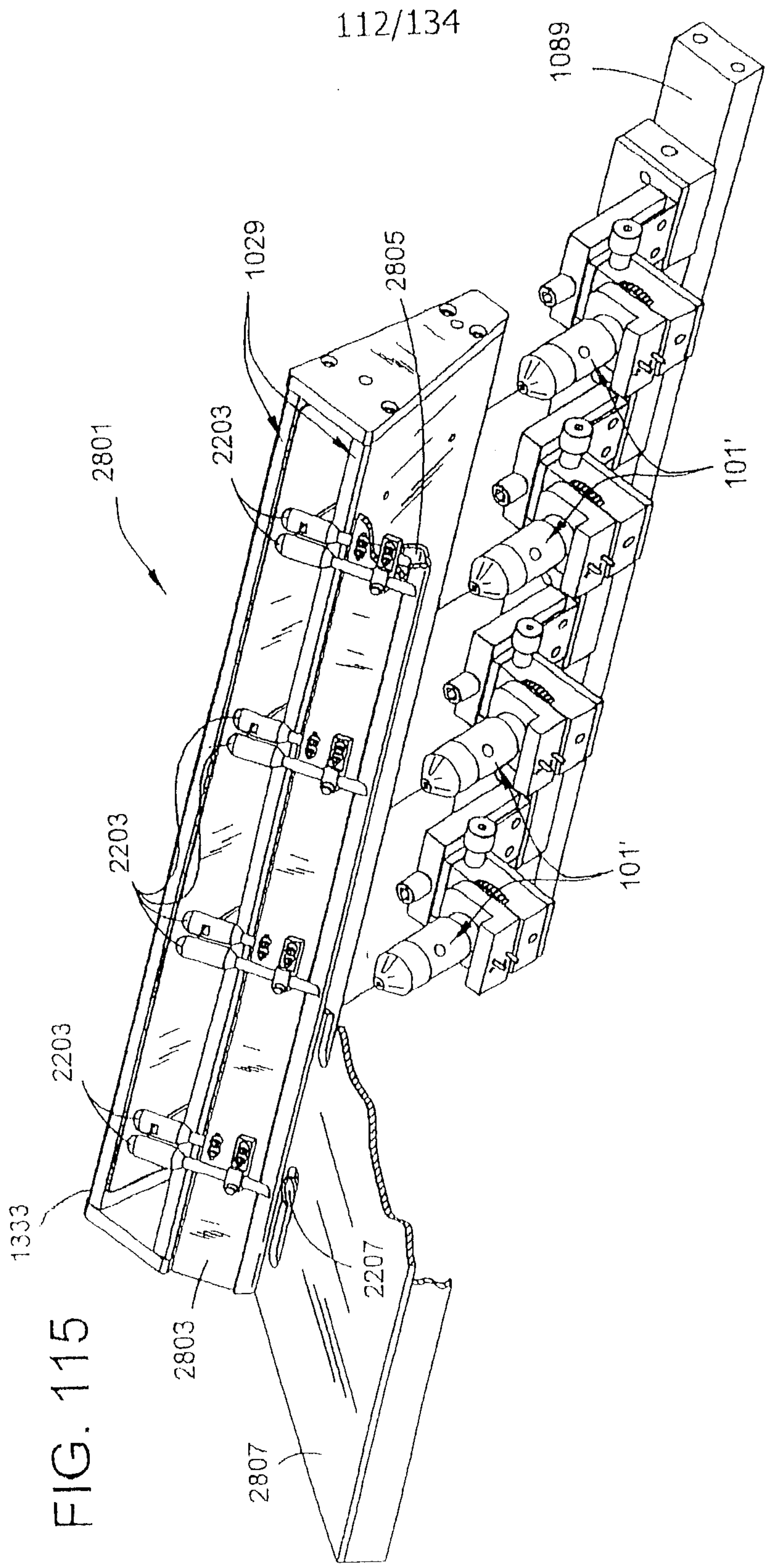


FIG. 116

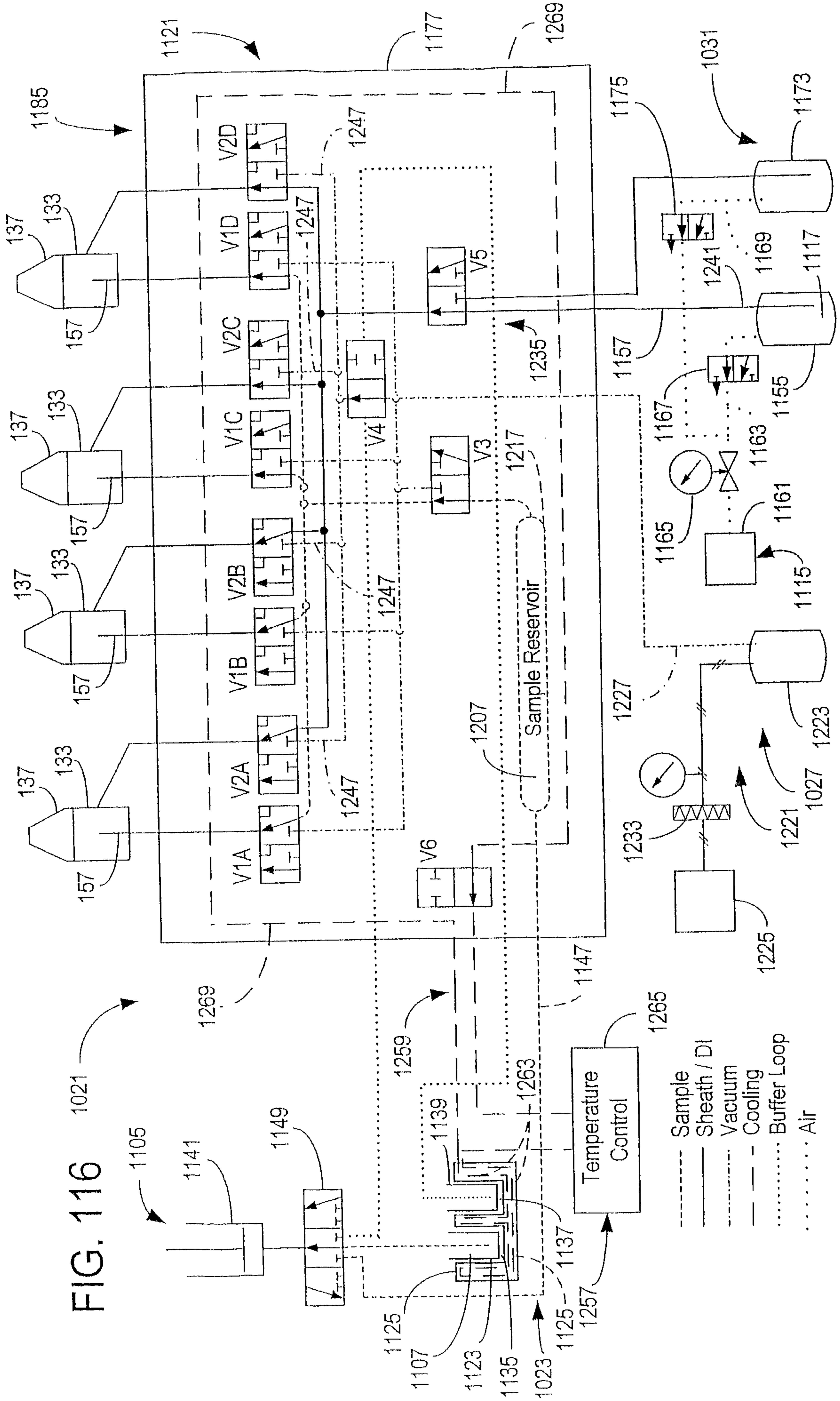


FIG. 117

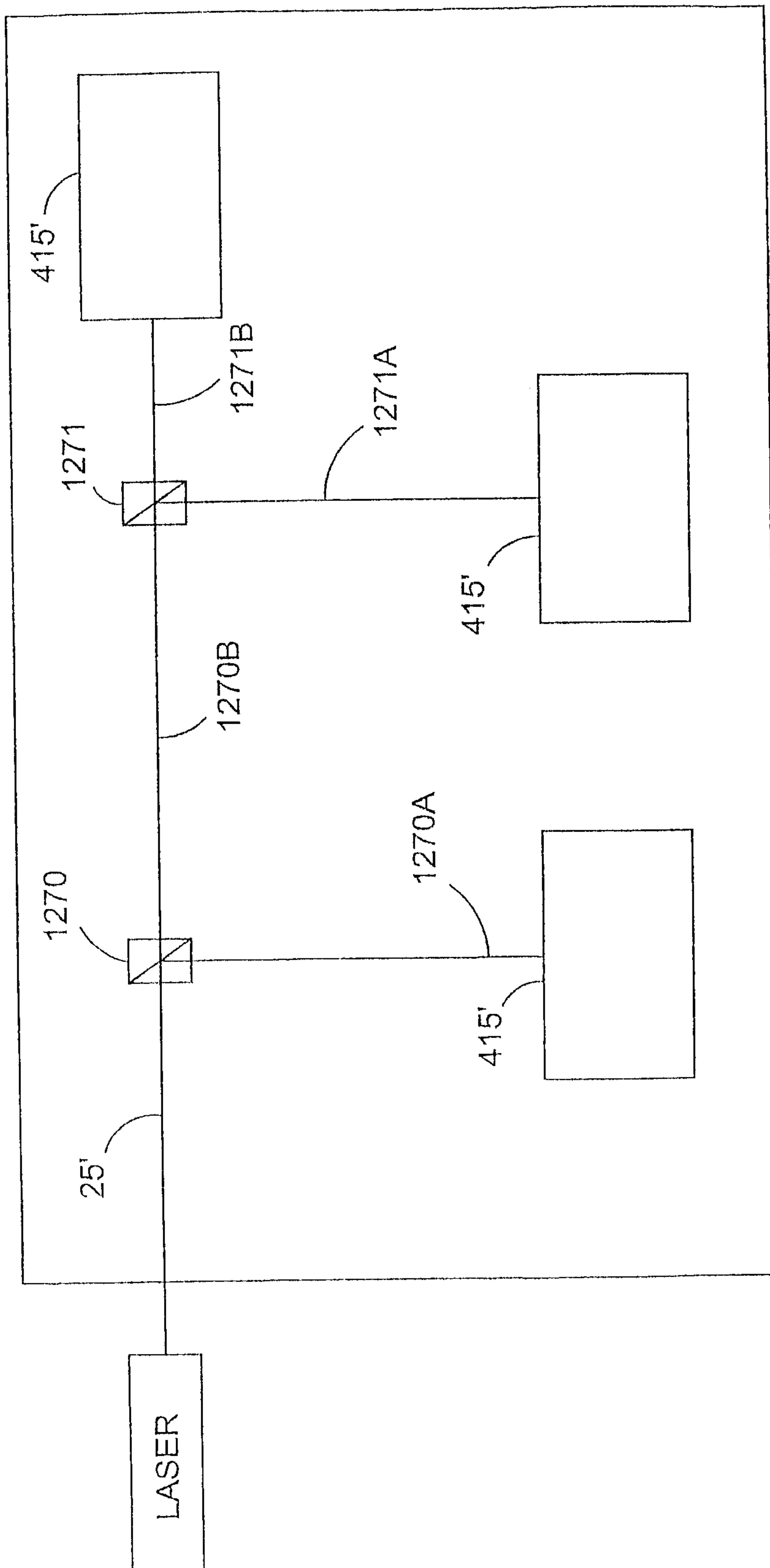
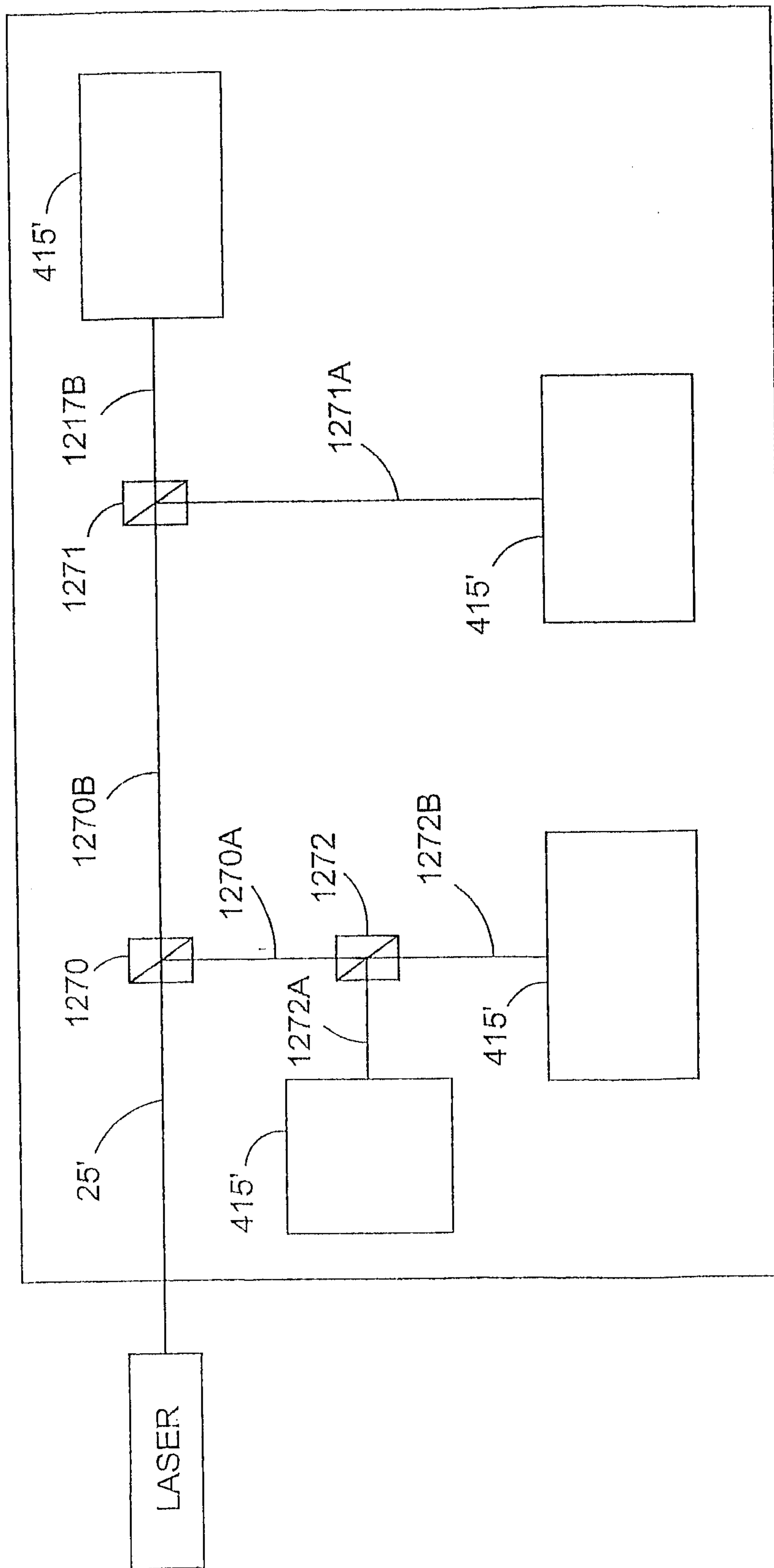


FIG. 118



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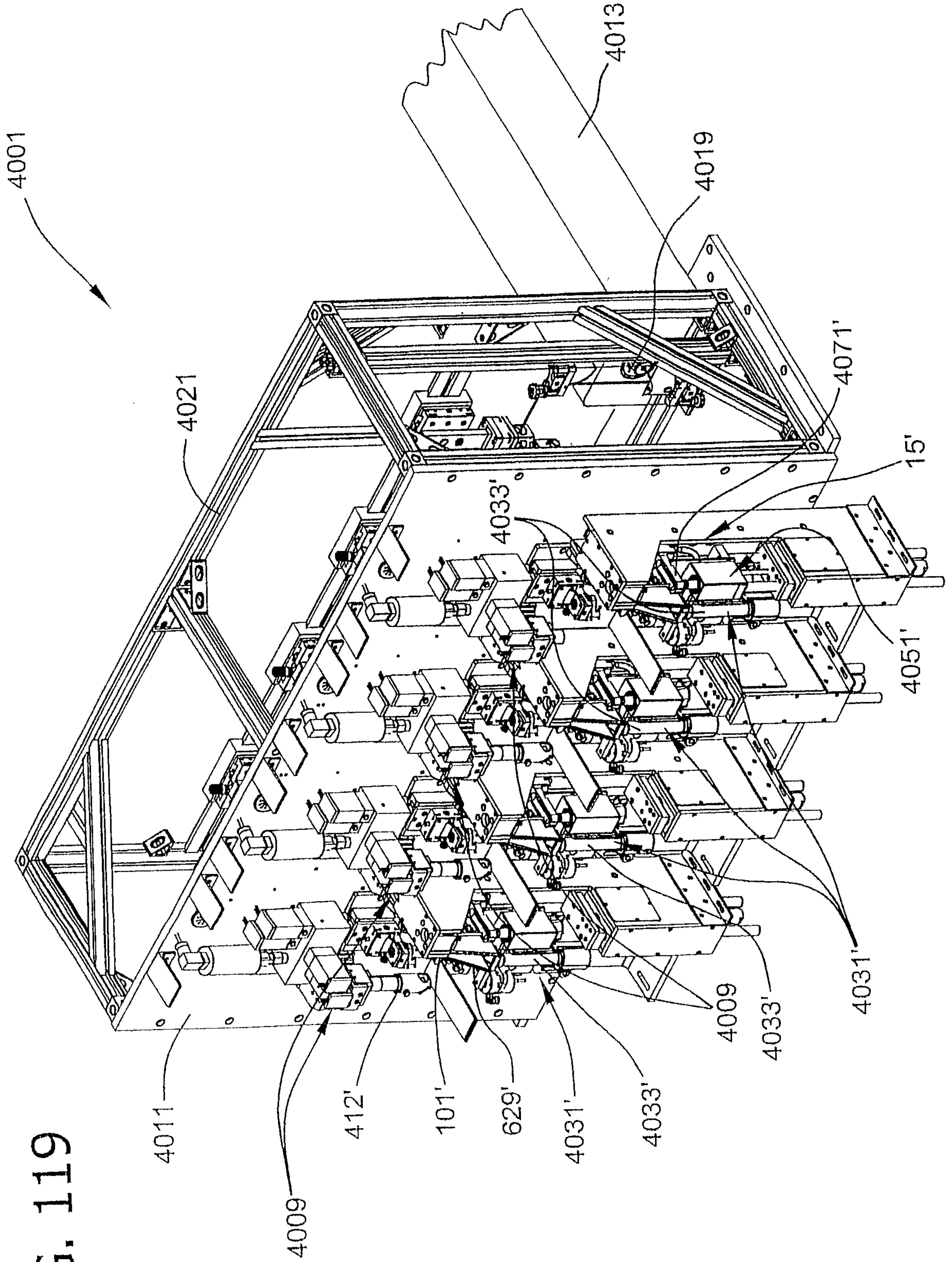


FIG. 119

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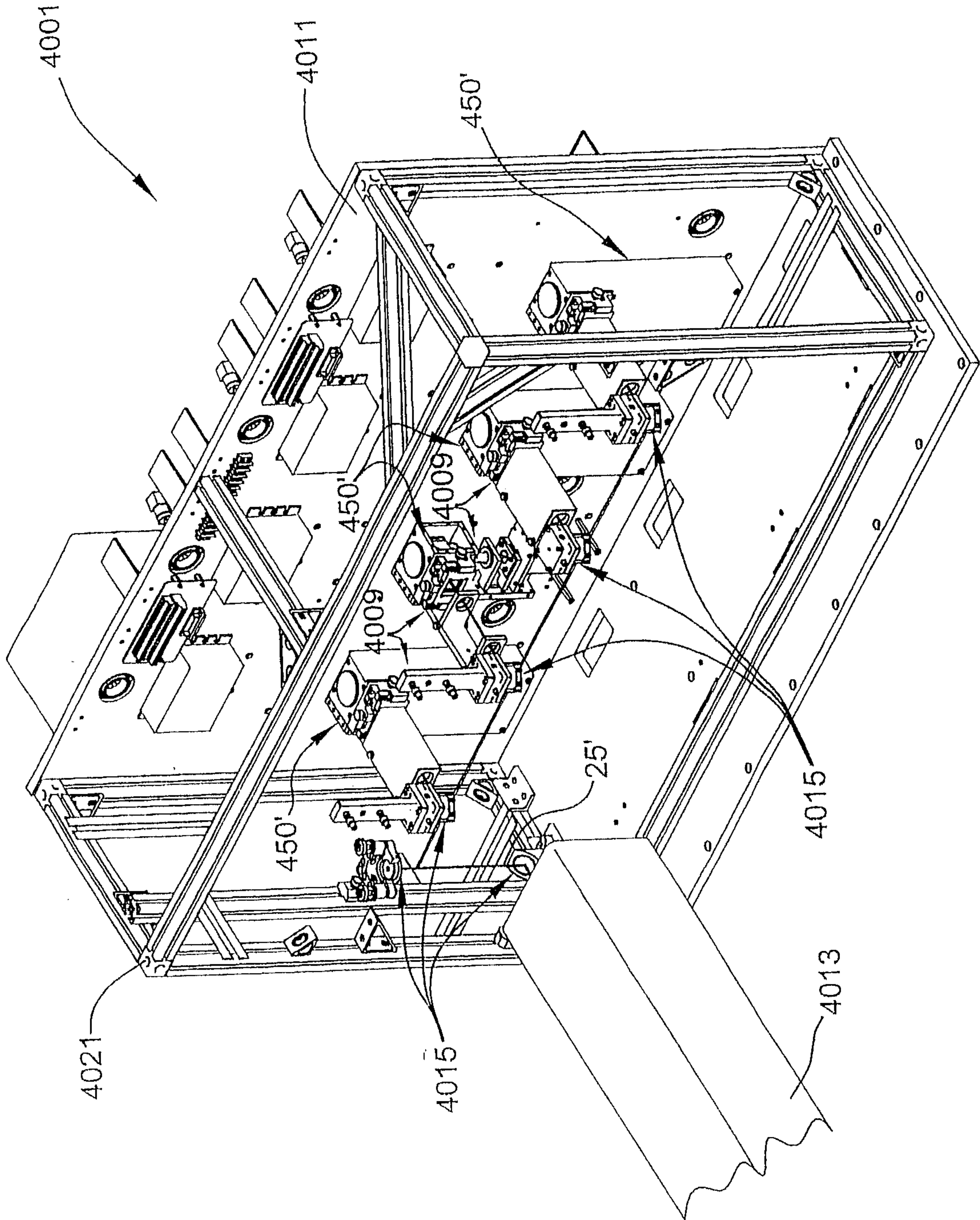
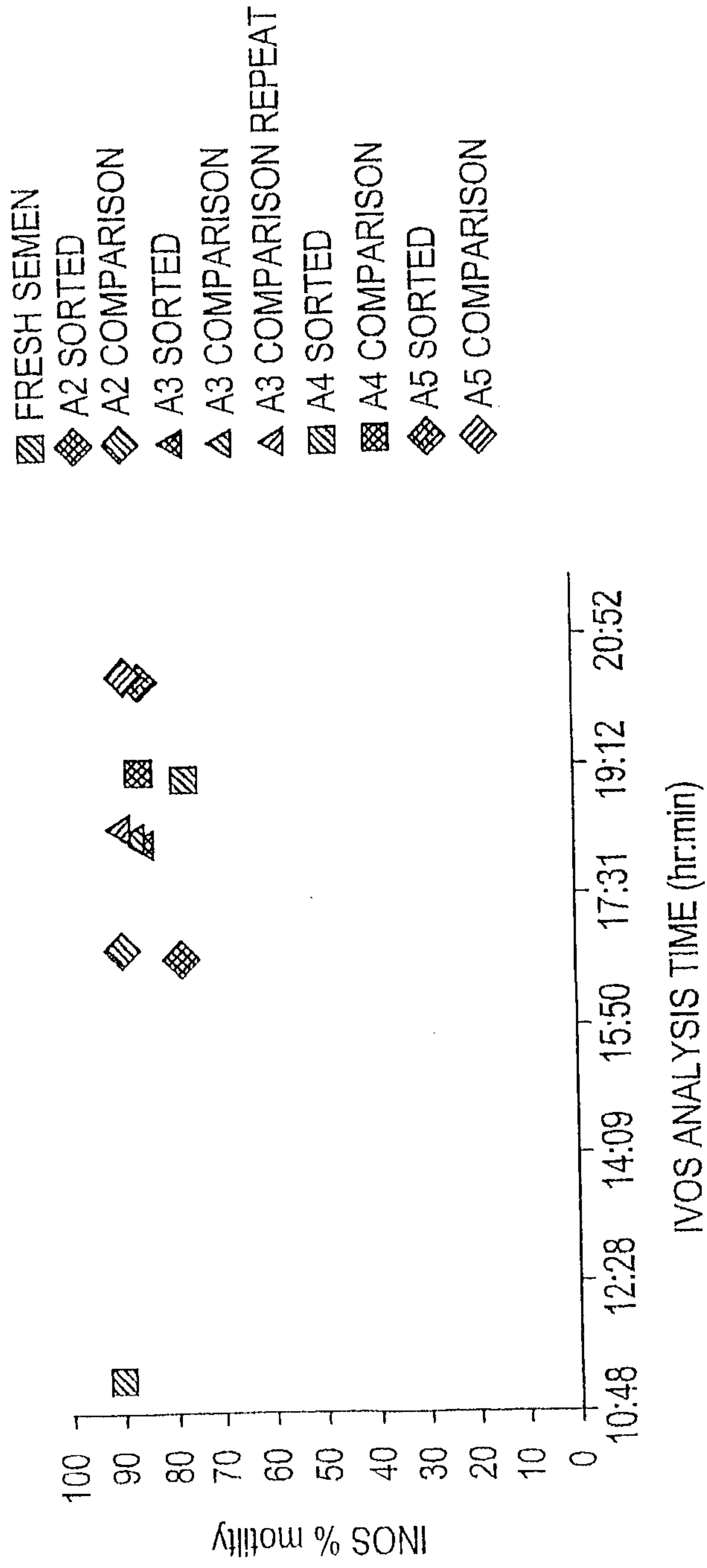


FIG. 120

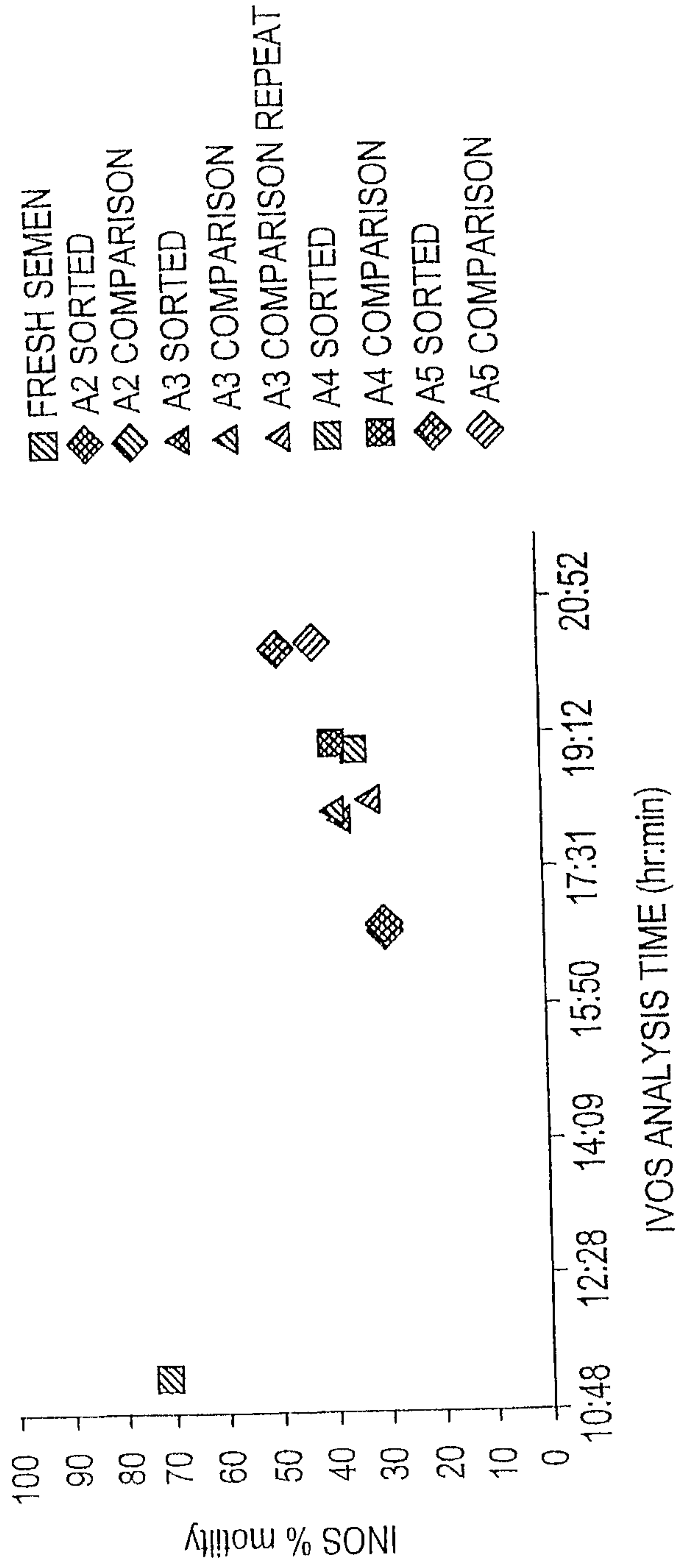
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FIG. 123

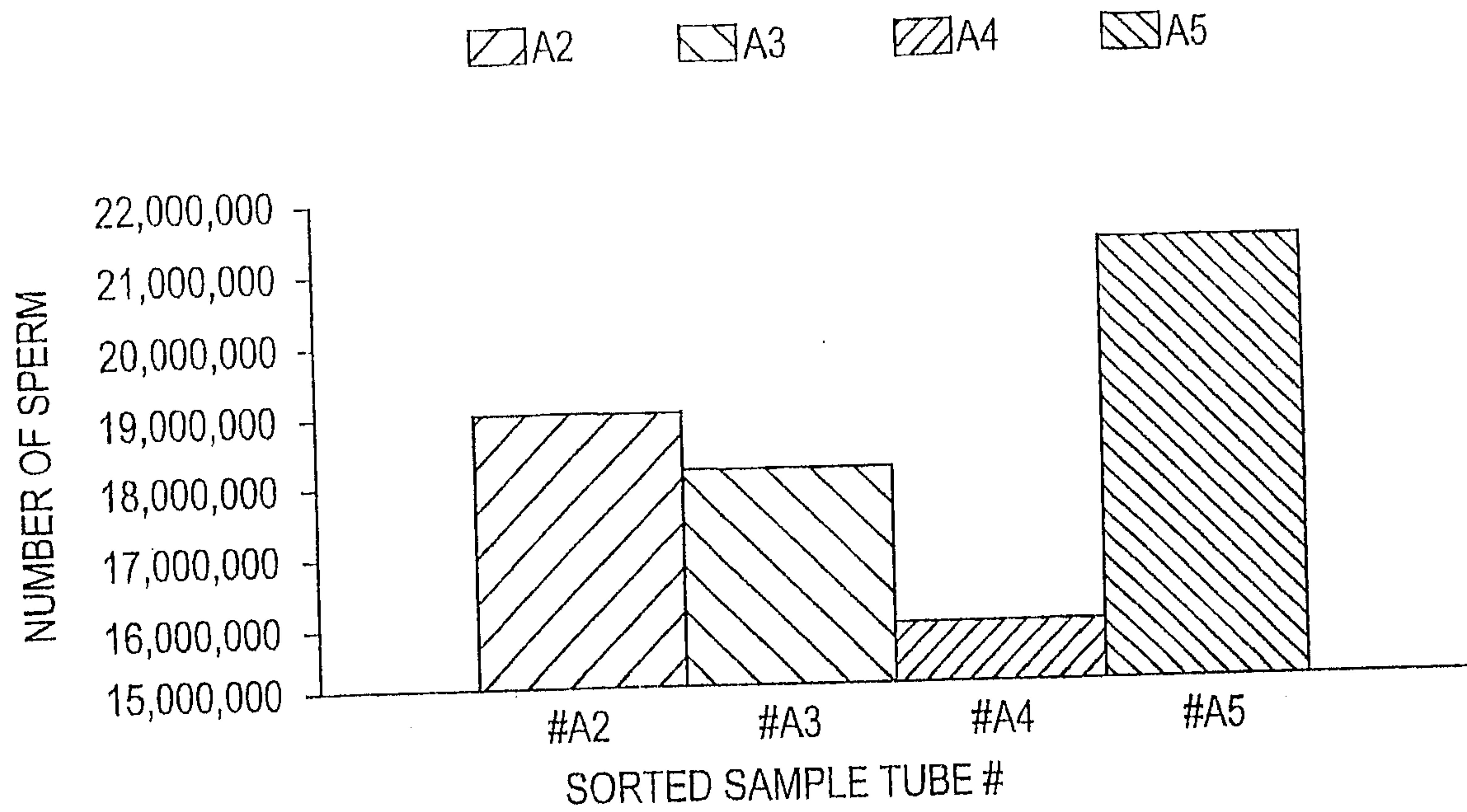
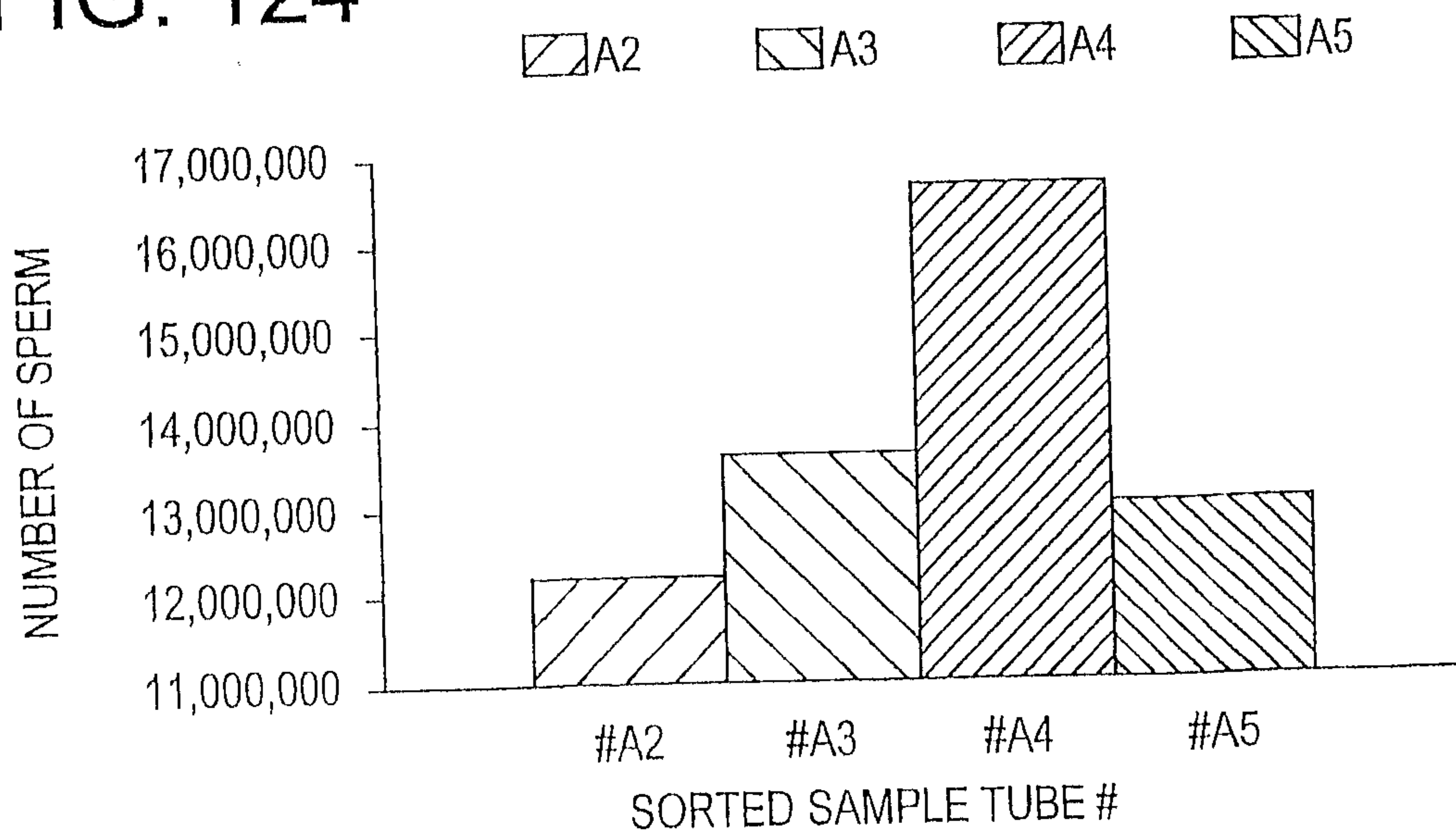
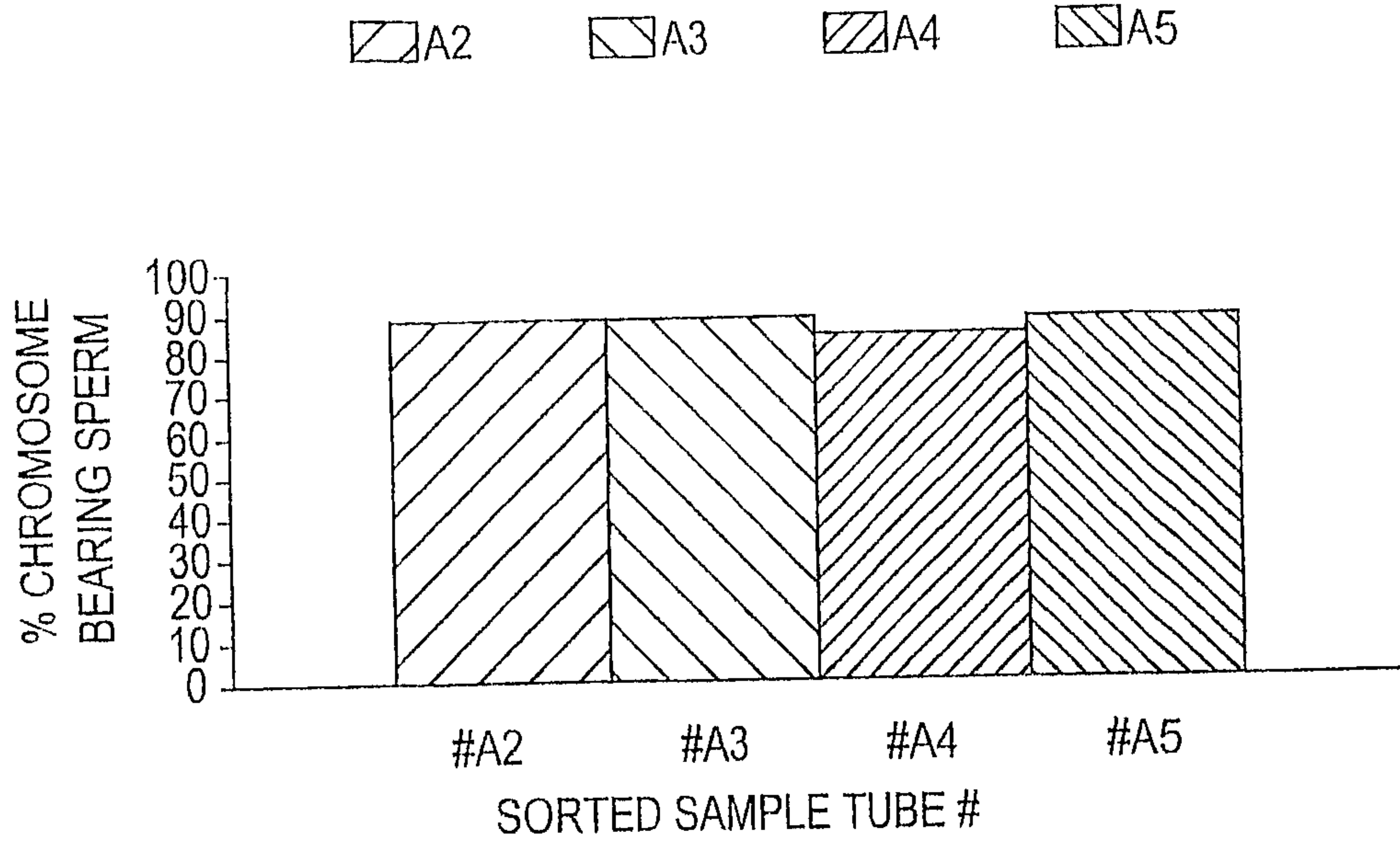


FIG. 124



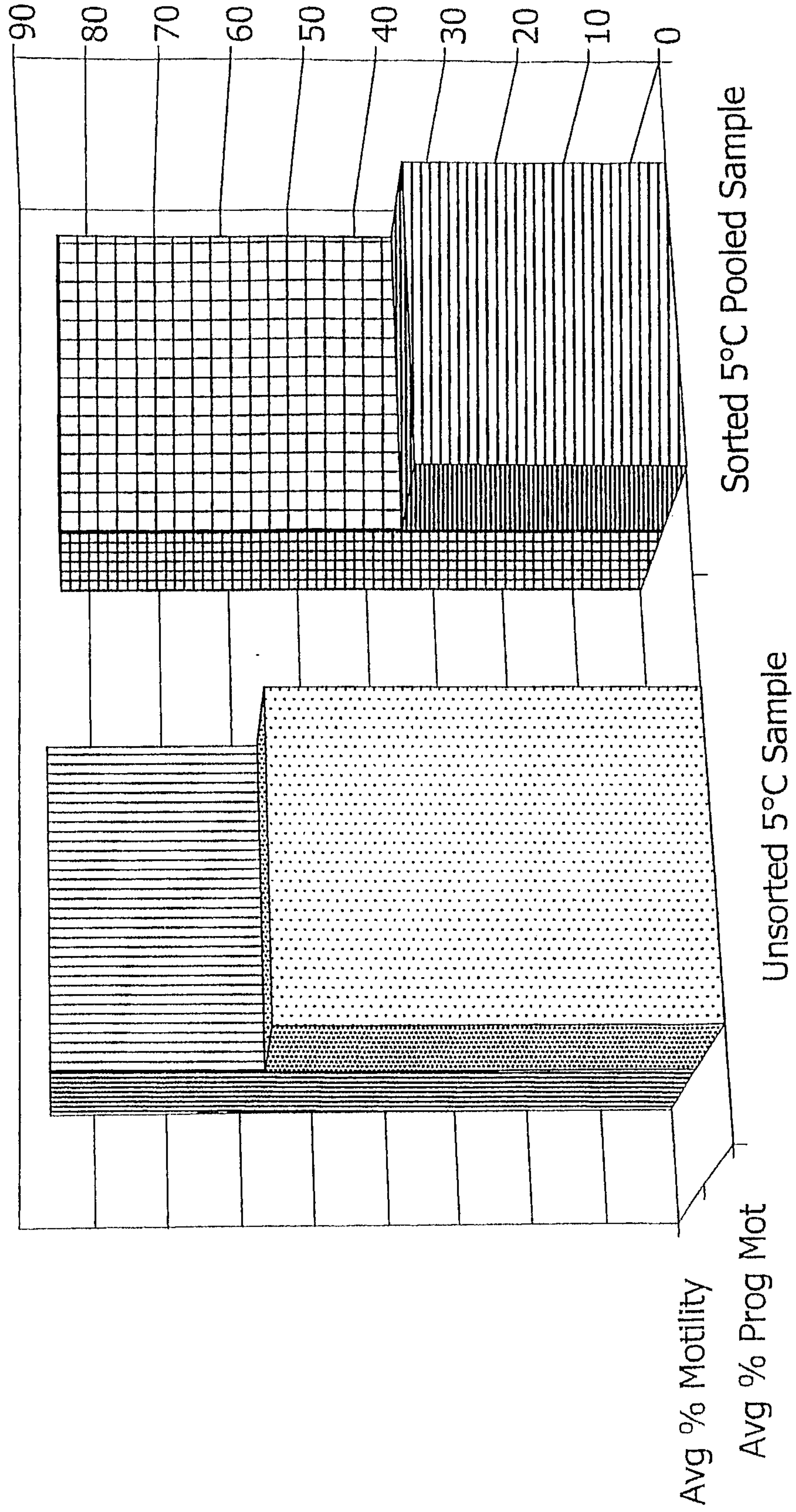
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FIG. 125



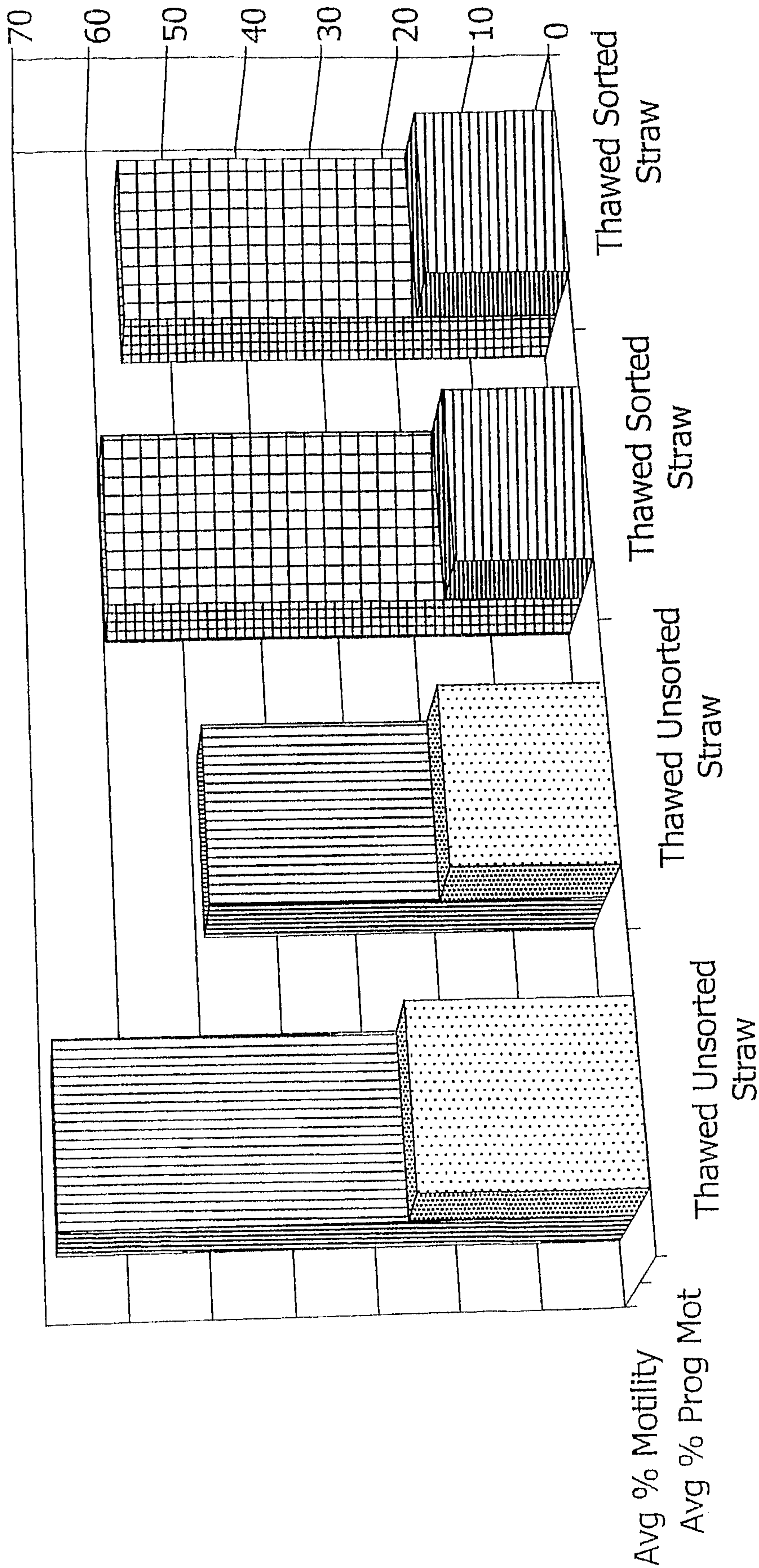
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FIG. 126



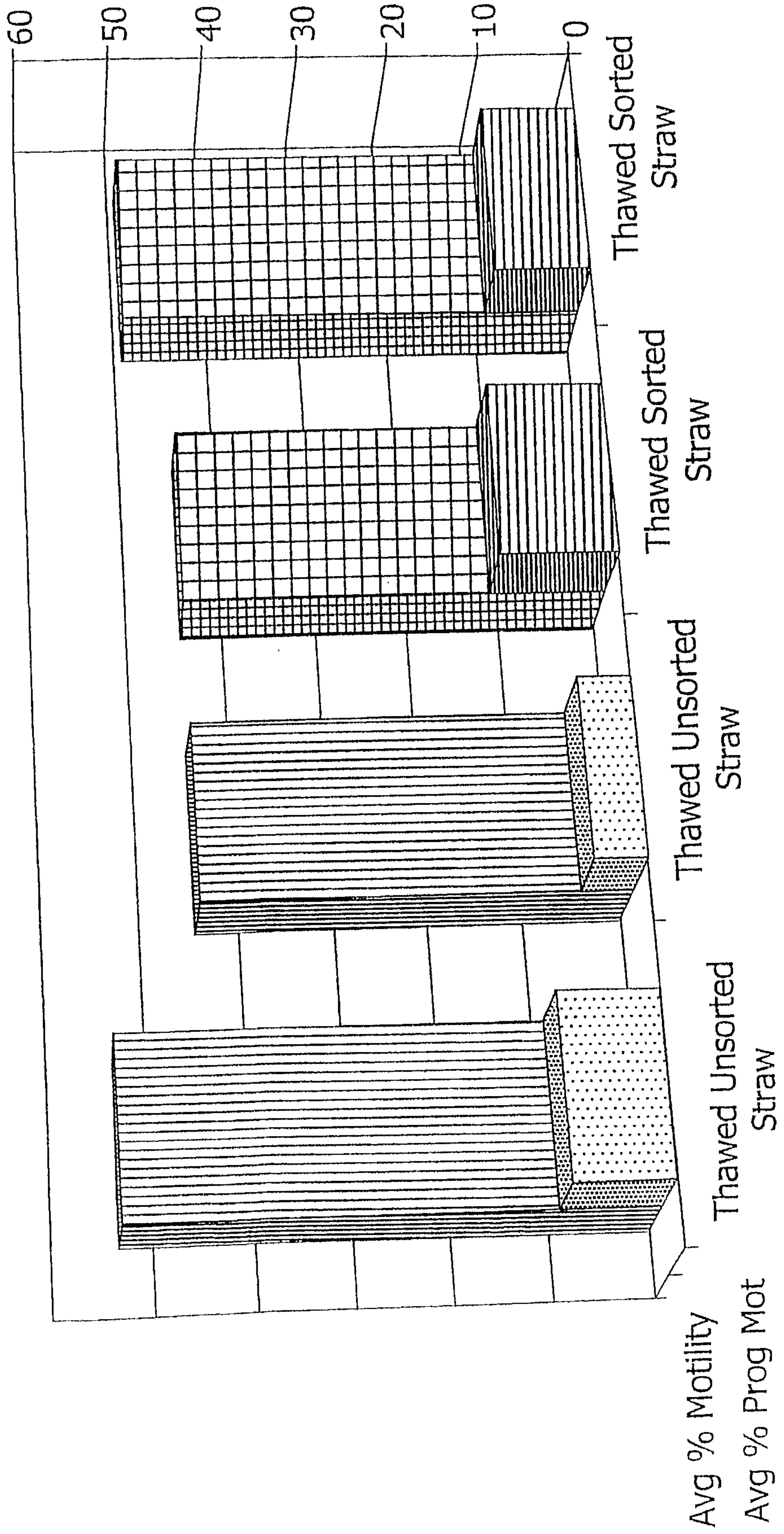
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FIG. 127



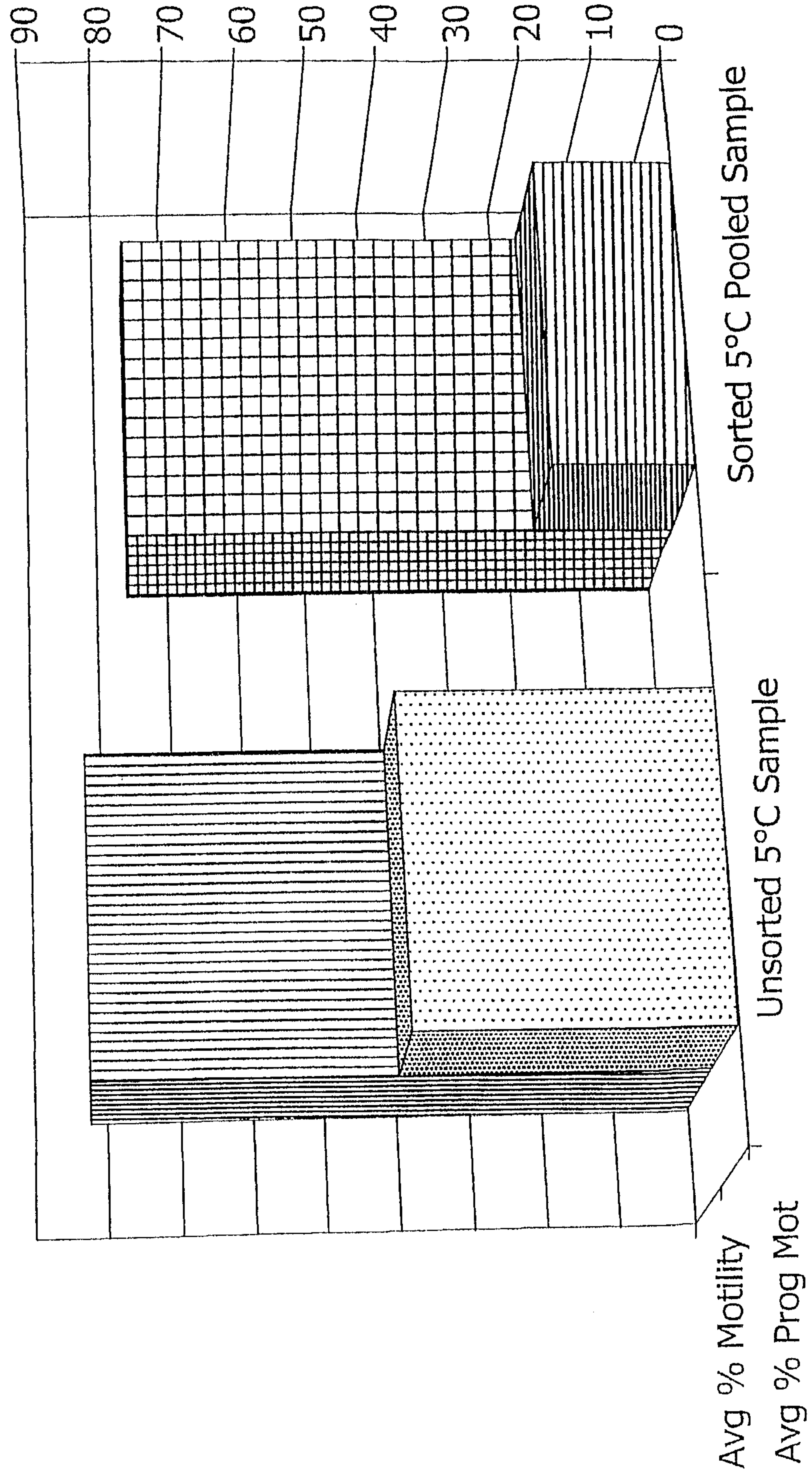
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FIG. 128



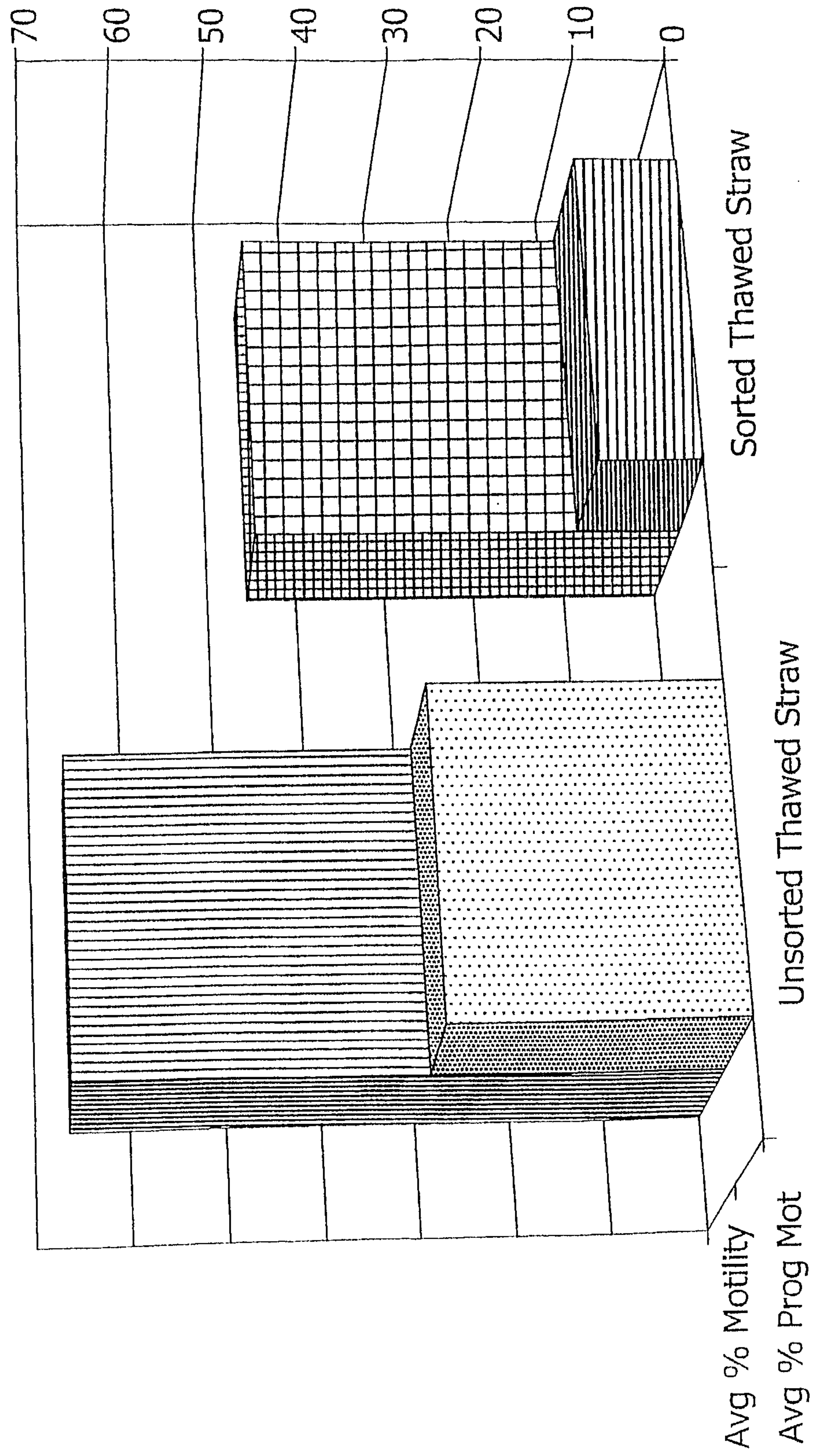
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FIG. 129



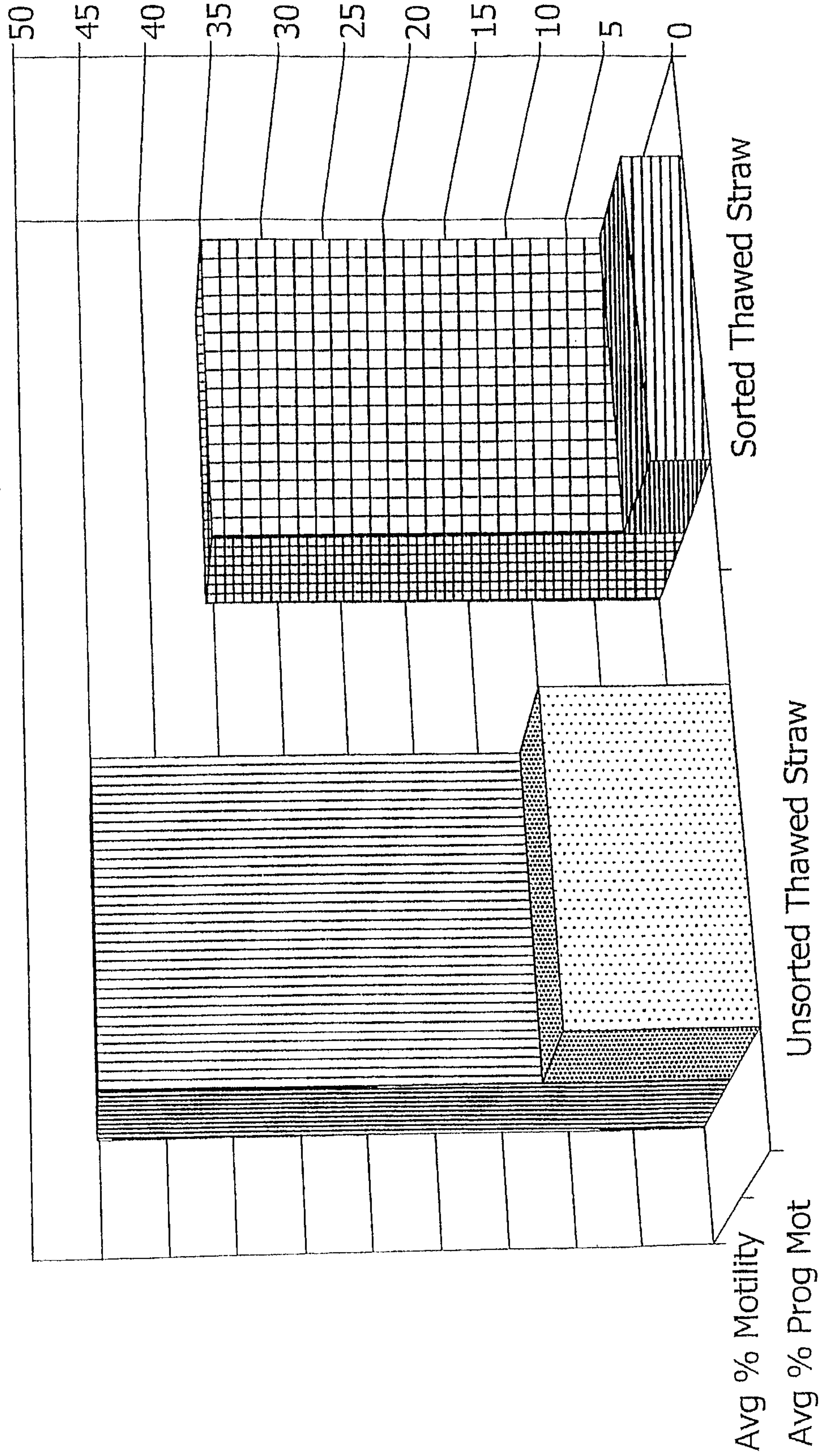
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FIG. 132

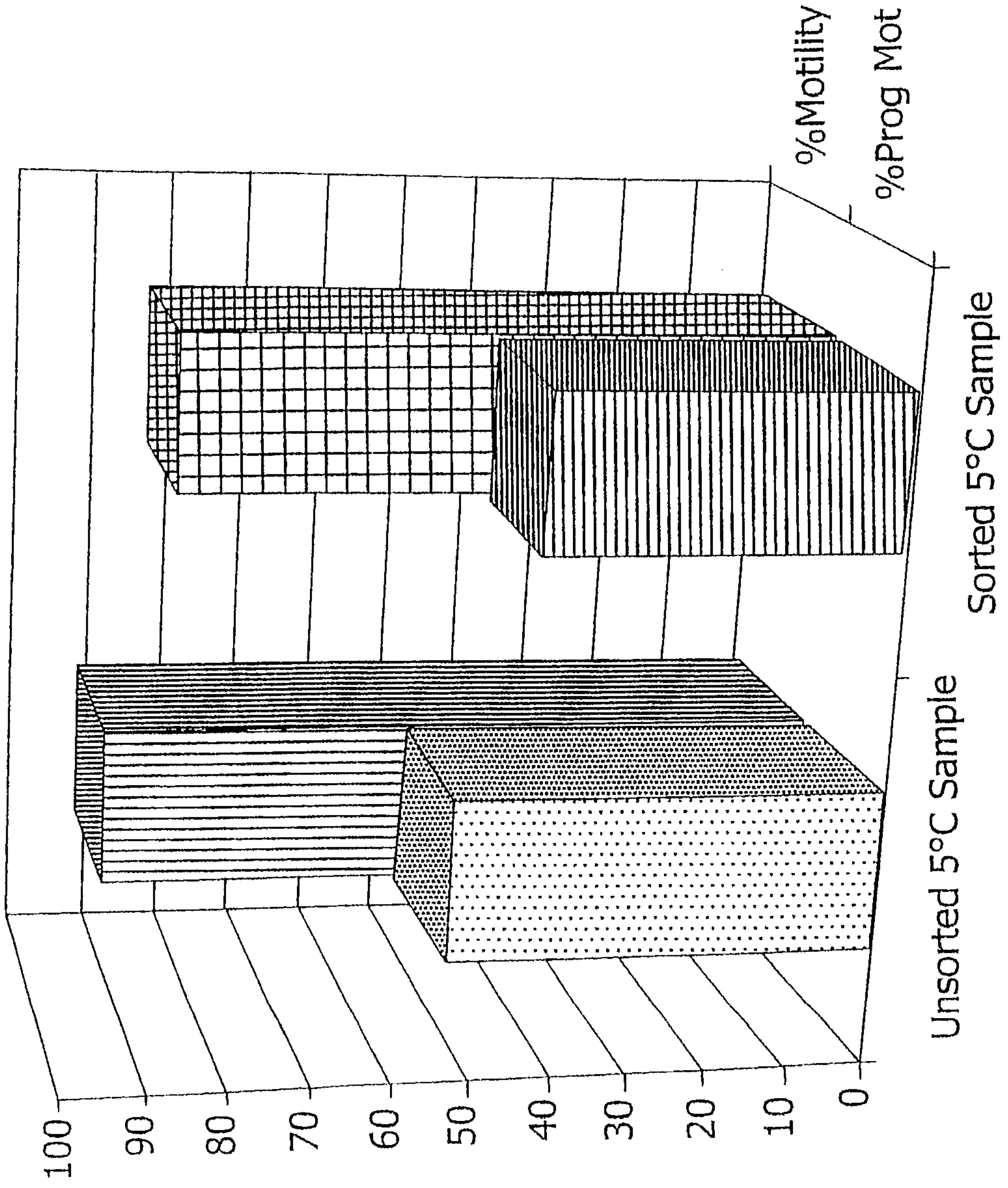
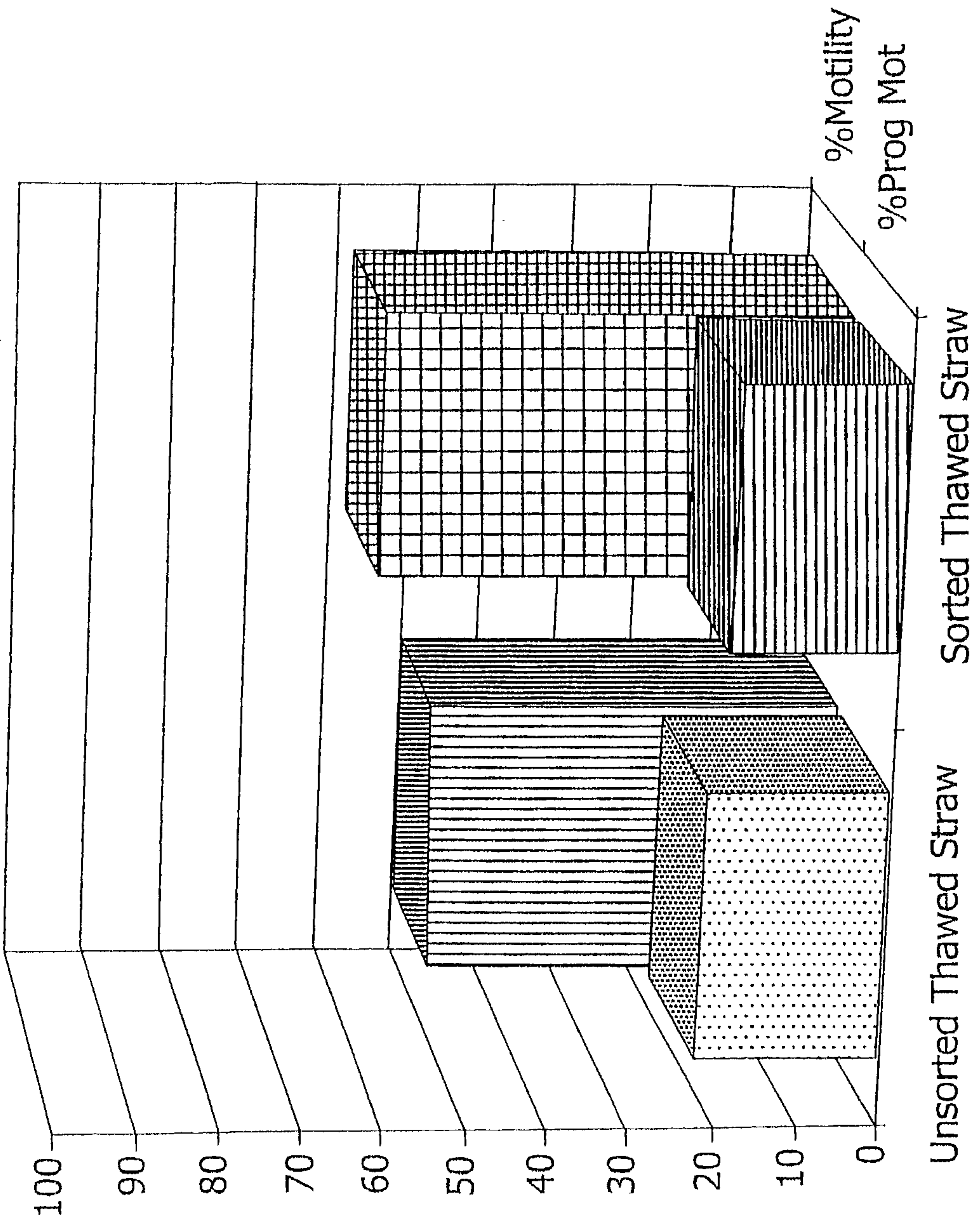
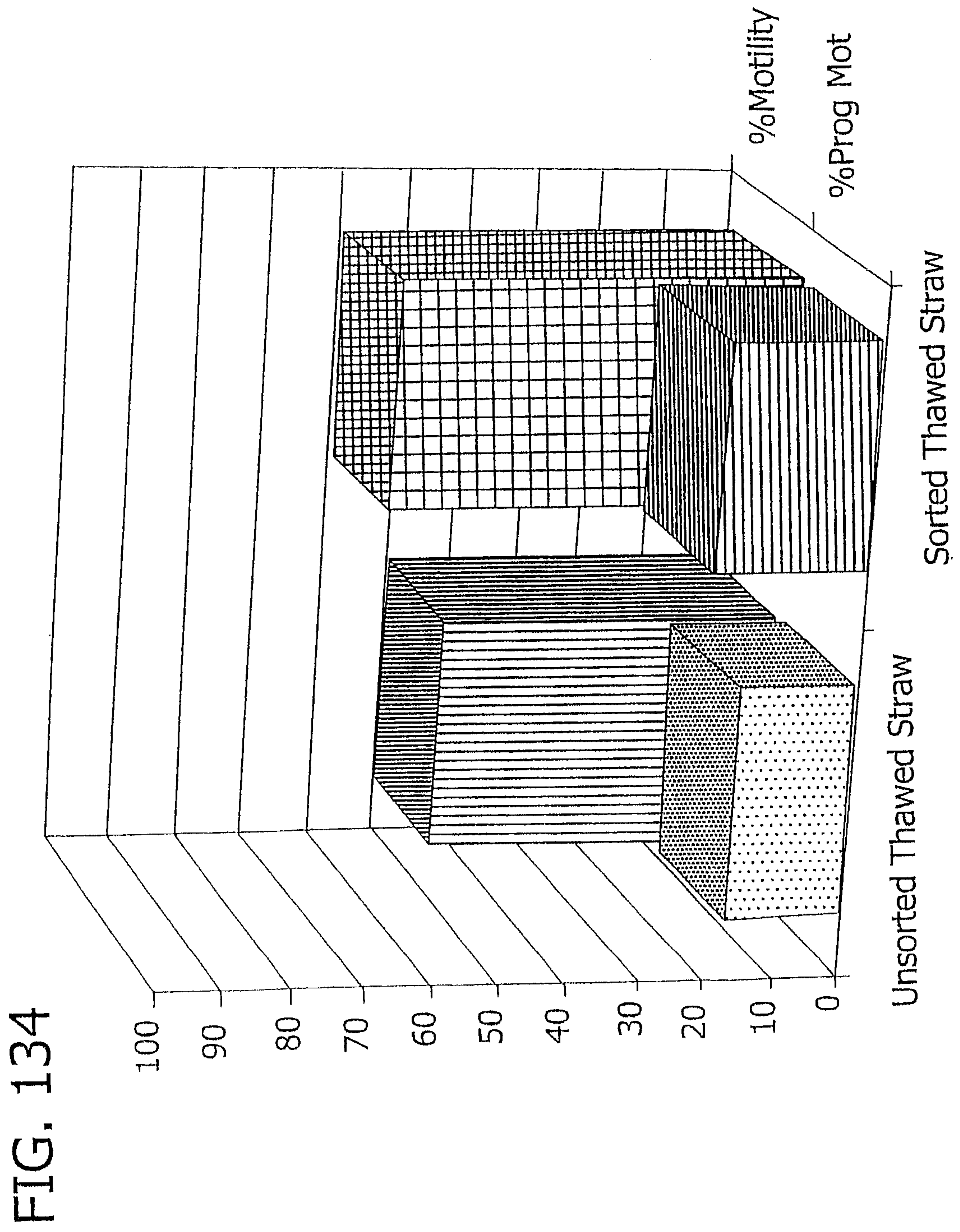


FIG. 133

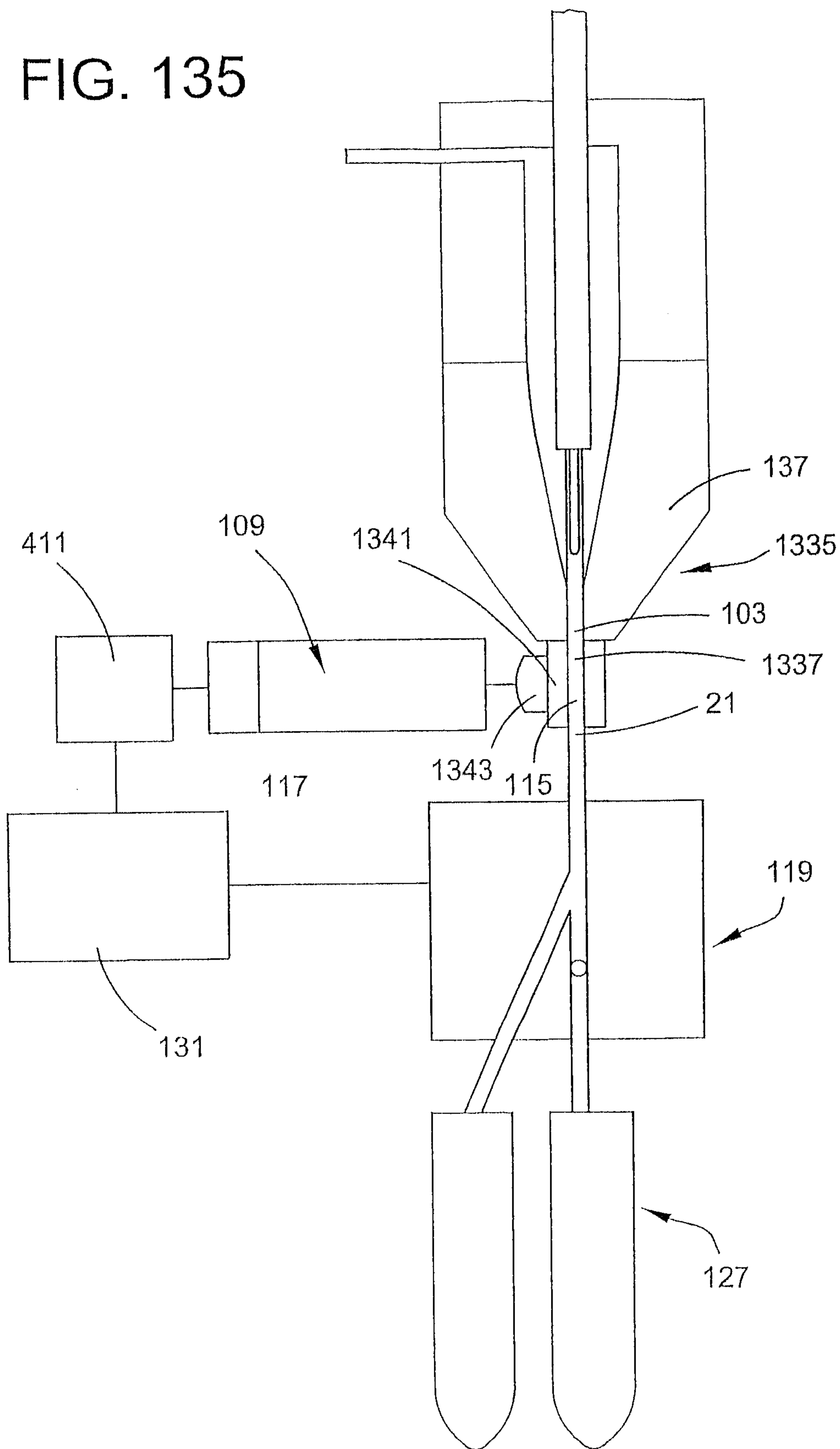


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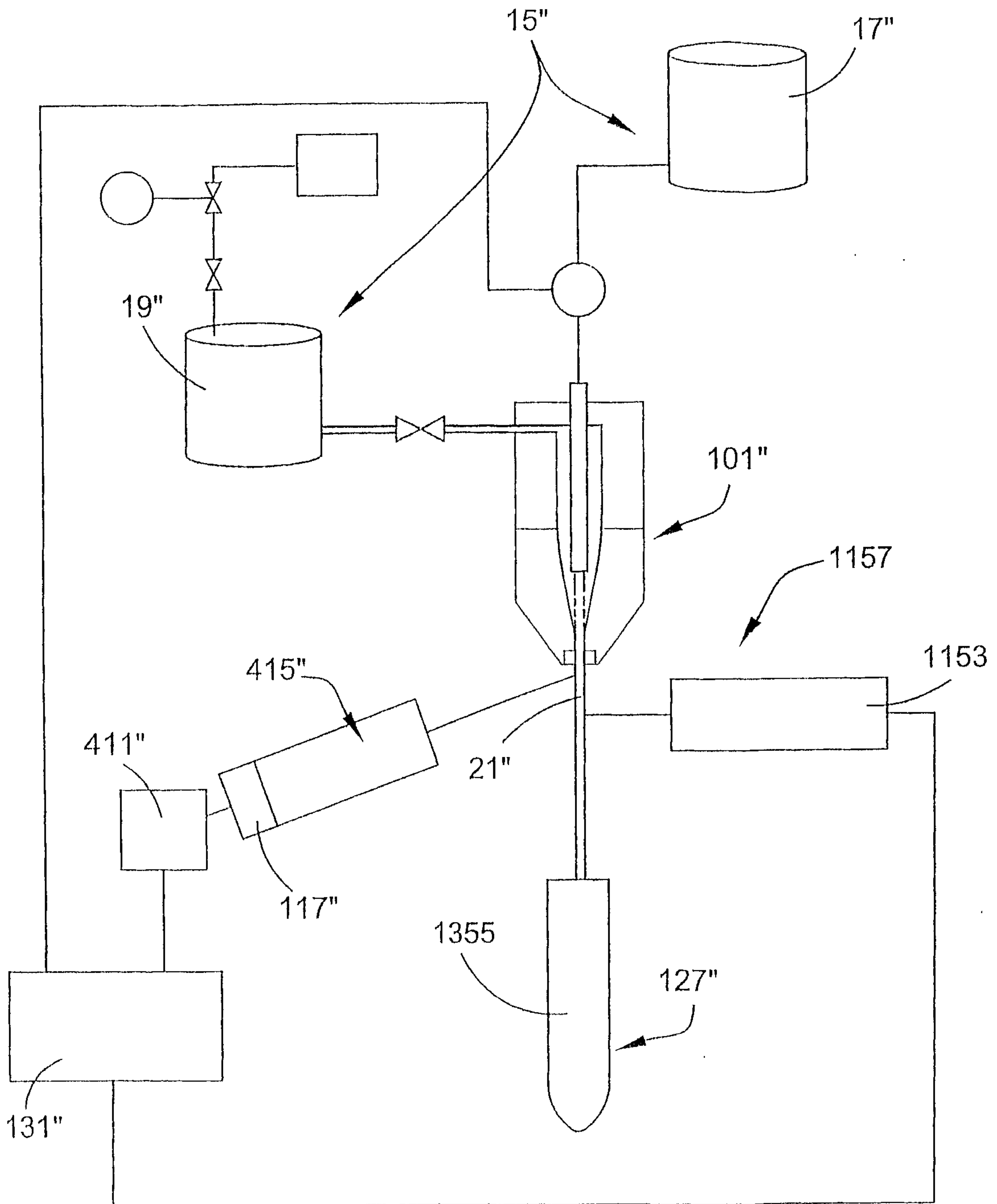
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FIG. 135



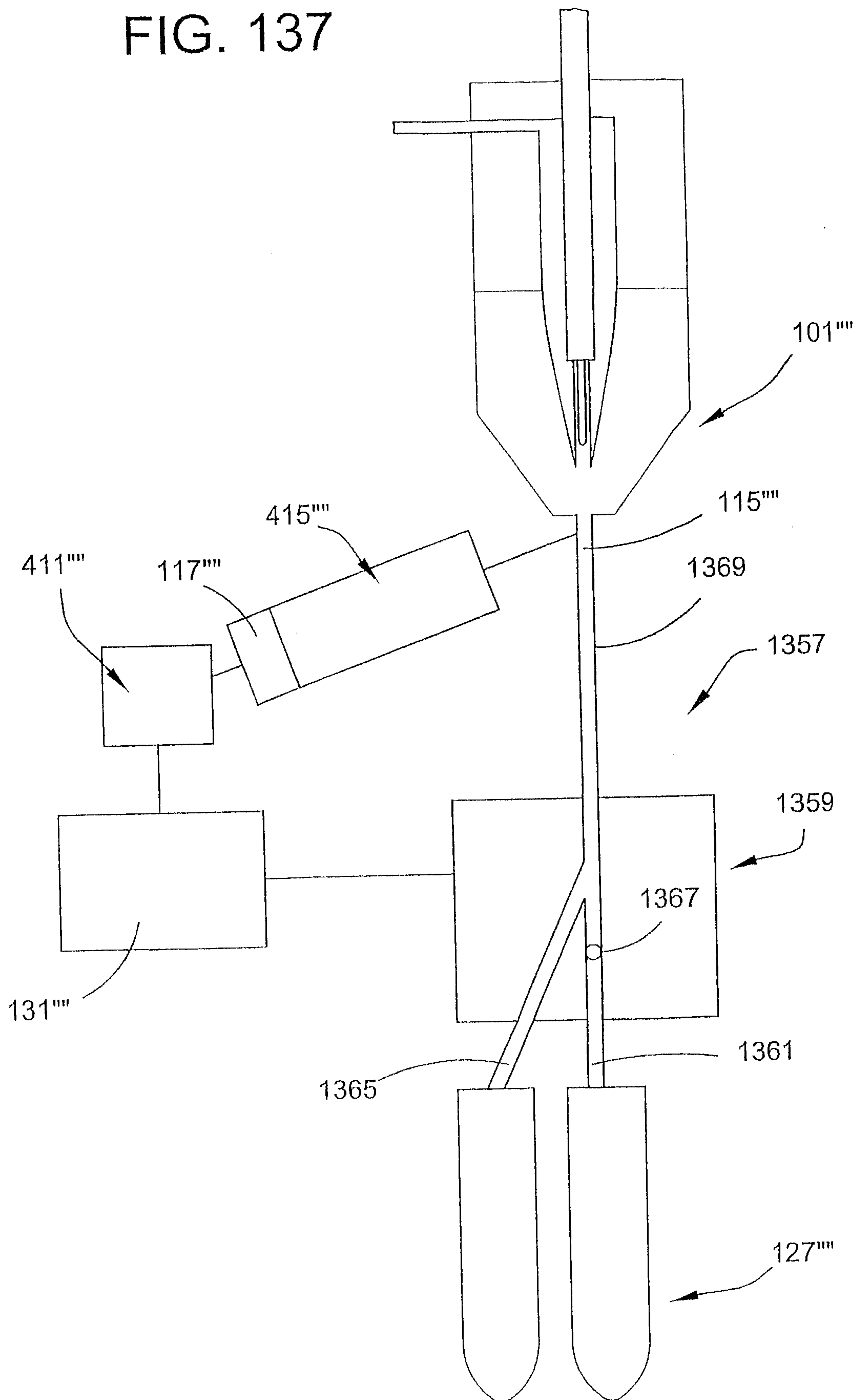
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FIG. 136



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FIG. 137



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FIG. 138

