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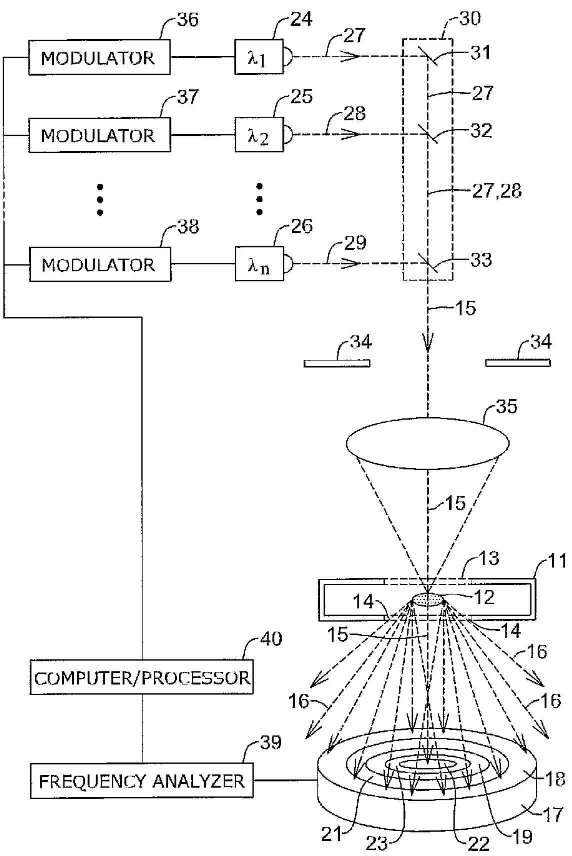
(54) Title: FREQUENCY-MULTIPLEXED DETECTION OF MULTIPLE WAVELENGTH LIGHT FOR FLOW CYTOMETRY



(57) Abstract: A multiplexed set of light sources having outputs of light with various wavelengths which are combined into one beam. The beam may impinge a particle in a flow channel of a cytometer. The light leaving the flow channel may be sensed by a detector and the light distinguished according to wavelength.

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FREQUENCY-MULTIPLEXED DETECTION OF MULTIPLE WAVELENGTH LIGHT FOR FLOW CYTOMETRY

Background

This invention pertains to cytometers and particularly to optical systems of cytometers. More particularly, the invention pertains to the optical acquisition of information about microscopic particles or components in a flow stream of a cytometer.

This invention is related to U.S. Patent Application Serial No. 10/225,325, by Bernard Fritz et al., filed August 21, 2002, and entitled "Optical Alignment Detection System", which is incorporated herein by reference; and the invention is related to U.S. Patent Application Serial No. 10/304,773, to Aravind Padmanabhan et al., filed November 26, 2002, and entitled "Portable Scattering and Fluorescence Cytometer", which is incorporated herein by reference. This invention also is related to U.S. Patent No. 6,549,275 B1, by Cabuz et al., issued April 15, 2003, and entitled "Optical Detection System for Flow Cytometry"; U.S. Patent No. 6,597,438 B1, by Cabuz et al., issued July 22, 2003, and entitled "Portable Flow Cytometer"; U.S. Patent No. 6,382,228 B1, by Cabuz et al., issued May 7, 2002, and entitled "Fluid Driving System for Flow Cytometry"; U.S. Patent No. 6,700,130 B2, issued March 2, 2004, by Fritz, and entitled "Optical Detection System for Flow Cytometry";

and U.S. Patent No. 6,240,944 B1, by Ohnstein et al., issued June 5, 2001, and entitled "Addressable Valve Arrays for Proportional Pressure or Flow Control"; all of which are incorporated herein by reference. The term "fluid" may be used herein as a generic term that includes gases and liquids as species. For instance, air, gas, water and oil are fluids.

Summary

The invention is an optical system for a cytometer using a multiplexing scheme to detect light of various wavelengths to obtain information relative to the particles that the light is impinging in a flow channel of the cytometer.

Brief Description of the Drawing

Figure 1 shows a multiplexed multiple wavelength light scattering system with a single detector; and

Figure 2 is a graph of light signals versus their respective modulation frequencies.

Figure 3 is a diagram of a cytometer as an illustrative example that may use the multiplexed multiple wavelength light scattering system.

Description

Improved performance (i.e., accuracy, selectivity, reliability, and so on) may be achieved by measuring optical scattering properties of a particle at multiple wavelengths. The invention may provide a way to accomplish this measuring approach by using a single detector assembly for all wavelengths. Each wavelength light source may be modulated at a unique frequency sufficiently separated from the other modulated sources to enable its signal to be demultiplexed unambiguously at the output of the detector. Light from all modulated sources scattered by the particle under measurement may be collected on the same detector assembly.

With flow cytometry, improved differentiation and accuracy in counting and distinguishing multiple particle types (e.g., blood cells) may be achieved by performing multi-dimensional measurements, such as particle volume, scattering at various angles, and scattering in various wavelengths. The invention may reveal improvements to this optical interrogation technique (i.e., multi-wave scattering). Scattering at multiple wavelengths may be done at spatially separated locations along the flow channel. This may require careful synchronization in timing as well as multiple detector arrays and spectra filters. This difficulty may be avoided by the use of modulation frequency multiplexing of the various wavelength sources. Each source may be modulated at a unique and sufficiently high frequency to meet system bandwidth requirements. The sources may be folded into one optical input path and focused simultaneously onto the same particle location. The scattered light at the various wavelengths may then be collected onto the same detector array to determine the angular information, and the signals at the different wavelengths may be separated by temporally filtering (e.g., Fourier transform methods) the detector signals.

Figure 1 shows an illustrative example implementing the invention. This figure shows a cross-section view of a channel 11. Channel 11 may be a flow or measurement channel of a cytometer. It may have a core stream having particles 12 moving through channel 11.

The core stream with particles 12 may be looked at as flowing into the surface of the figure. Channel 11 may be lengthy. The core stream along with particles 12 may be kept away from the inside surfaces of channel 11 with a sheathing fluid that surrounds the core stream. The location of the cross-section of channel 11 may be where a light source and detector arrangement may be placed. Channel 11 may have transparent windows 13 and 14 to facilitate the light source detector arrangement. A light beam 15 may enter channel 11 through window 13, impinge a particle 12 which may scatter beam 15 into light 16 which may exit channel 11 through window 14.

Light 16 may be sensed by a detector 17. Detector 17 may be an annular type having a ring of surface area 18 sensitive to light. The detector 17 may be expanded with another ring of surface area 19 also sensitive to light 16. Light sensitive surfaces 18 and 19 may be isolated form each other by an annular area 21 that is not sensitive to light. Also, detector 17 may be further expanded with a central light-sensitive area 22 that may be isolated from the light-sensitive annular area 19 by an annular area 23 that is not sensitive to light. The detector 17 may be expanded to include as many annular detectors, each subtending its own prescribed angular interval, as needed. The annular detectors or other kinds of detectors of an array of the detector may provide electrical signals representing light impinging the detector at respective angles. That is, one electrical signal may represent detected light of a first angle; another electrical signal may represent detected light of a second angle; and so on.

Various kinds of information may be obtained about the particles 12 from the scattered light. First, a count of the particles 12 may be made with the successive interruption of the light beam 15 to detector 17. Other information about the size, shape, surface, and so on, about particles 12 may be obtained from scattered light that impinges detector 17. The magnitudes of the scattered light and the location of such light on detector 17 may be noted electronically from the signals from the various detector 17 surfaces. Another dimension of information may be obtained from the scattered light if the various wavelengths of the scattered light are known. Light 15 beams of various wavelengths may scatter differently from particles 12. That is, a light beam of one wavelength may scatter differently than a light beam of another wavelength for the same point of impingement of a particle, or even the same particle, in the same location. These differences of scattering may provide additional information about the particle.

To accomplish projecting a light beam 15 having various but identifiable frequencies of light may be achieved with the present invention. Beam 15 may be composed of light from a number (n) of light sources 24, 25 and 26. Light source 24 may emit or emanate a light beam 27 having a wavelength λ_1. Light source 25 may emanate a light beam 28 having a wavelength λ_2, and light source 26 may emanate a beam 29 having a wavelength λ_n. Between light source 25 and light source 26 may be numerous similar light sources with light beams having different wavelengths, respectively.

Beam 27 may propagate from source 24 to a component dichroic mirror 31 in a dichroic fold mirror assembly 30. Mirror 31 may reflect at least a portion of beam 27 approximately 90 degrees towards channel 11. Beam 28 may propagate to a dichroic mirror 32 of assembly 30. Mirror 32 may deflect and/or reflect at least a portion of beam 28 approximately 90 degrees towards channel 11. Beam 29 may propagate to a dichroic mirror 33 of assembly 30. Mirror 33 may reflect at least a portion of beam 29 approximately 90 degrees towards channel 11. There may be additional beams and mirrors between beams 28 and 29 and between mirrors 32 and 33, respectively.

As beam 27 propagates toward channel 11, it may, at least in part, go through mirrors 32 and 33 and any additional mirrors between those mirrors. Likewise, as beam 28 propagates toward channel 11, it may, at least in part, go through mirror 33 and any mirrors between mirrors 32 and 33. A resultant beam 15, which may include beams 27, 28 and 29 and any beams reflected or deflected by other mirrors situated between mirrors 32 and 33 of assembly 30. Beam 15 may proceed through aperture 34, optics 35 and window 13 of channel 11.

Since beam 15 may go through window 13 of channel 11, impinge a particle 12 and be scattered as light beams 16 that go through window 14 to the detector 17, there may be an interest to determine which wavelengths each of the light beams 16 has. The answer might not be evident in how to identify the wavelength or source of the reflected light in the electrical signals being output from detector 17.

To identify the wavelength of the detected light 16, scattered or unscattered, may be achieved with modulation of the light from each of the sources. That is, a modulator 36 may modulate the output of the light source 24 with a frequency f_1. Also, a modulator 37 may modulate the output of light source 25 with a frequency f_2 and modulator 38 may modulate the output of light source 26 with a frequency f_n. Between modulators 37 and 38 there may be other modulators that modulate additional light sources of other wavelengths that may be situated between light sources 25 and 26. This approach may be regarded as a frequency multiplexing of the light sources. Modulators 36, 37, 38 and the other modulators may be connected to and controlled by computer/processor 40.

The output of detector 17 may go to a frequency analyzer 39 which may demultiplex the detected light 16 and 15 signals and separate out the light into component signals according to their wavelengths and respective light sources. These signals may be provided to the computer/processor 40 for analysis, counting, identification, recording and/or other actions.

Modulation frequencies may be relatively high in comparison to signal frequencies. Figure 2 reveals a graph of the signals multiplexed according to frequency.

As an illustrative example, a signal 41 may be of the wavelength λ_1 multiplexed at 10.0 MHz, a signal 42 may be of the wavelength λ_2 multiplexed at 10.3 MHz, and a signal 43 may be of the wavelength λ_n multiplexed at 10.6 MHz. Additional signals of other wavelengths may be multiplexed at other frequencies for demultiplexing at the output of the detector 17.

Figure 3 is a diagram of a cytometer 45 that may incorporate an illustrative application of the multiplexed multiple wavelength light scattering system. Cytometer 45 may have a channel 11 with a core stream of particles 12.

Although the invention has been described with respect to at least one illustrative embodiment, many variations and modifications will become apparent to those skilled in the art upon reading the present specification. It is therefore the intention that the appended claims be interpreted as broadly as possible in view of the prior art to include all such variations and modifications.

What is claimed is:

1. A multiple wavelength light system comprising:

a plurality of light sources;

an at least one mirror proximate to the plurality of light sources;

at least one multiplexer connected to the plurality of light sources;

a detector proximate to a target; and

a demultiplexer connected to the detector.

2. The system of claim 1, further comprising a processor connected to the at least one multiplexer and the detector.

3. The system of claim 2, wherein:

the at least one multiplexer is a frequency modulator; and

the processor comprises a frequency analyzer.

4. The system of claim 3, wherein the detector has light sensitive sections to detect light at various angular intervals.

5. The system of claim 1, further comprising a demultiplexer connected to the detector.

6. The system of claim 5, further comprising a processor connected to the at least one multiplexer and the demultiplexer.

7. A multiple wavelength light system comprising:

a first light source having a first wavelength;

a second light source having a second wavelength;

a first modulator, having a first modulation frequency, connected to the first light source;

a second modulator, having a second modulation frequency, connected to the second light source;

a light combiner proximate to the first and second light sources;

a detector mechanism proximate to an output of the light combiner; and

a frequency demodulator connected to the detector.

8. The system of claim 7, wherein the detector mechanism is an array of detectors.

9. The system of claim 7, further comprising:

a third light source having a third wavelength; and

a third modulator, having a third modulation frequency, connected to the third light source; and

wherein the light combiner is proximate to the third light source.

10. The system of claim 9, wherein:

an output of the light combiner is focused on a target; and

the detector mechanism is proximate to the target.

11. The system of claim 10, wherein a signal from the detector mechanism can be broken out according to light source.

12. The system of claim 10, wherein an output signal from the detector mechanism may be demultiplexed according to wavelength of the light impinging the detector mechanism.

13. A multiple wavelength optical system comprising:

a plurality of light sources;

a frequency modulator connected to each light source;

a dichroic fold mirror having an element proximate to each light source, and an output of light directed to a target;

a light detector proximate to the target; and

a frequency analyzer connected to the light detector.

14. The system of claim 13, wherein:

at least one light source of the plurality of light sources emits light having a wavelength different than the wavelength of light emitted by another light source; and

the at least one light source emits light modulated with a frequency different from a frequency that light emitted by another source is modulated with.

15. The system of claim 14, further comprising:

a frequency analyzer connected to the detector; and

wherein signals caused by light emitted by the at least one light source of one wavelength of the plurality of light sources are distinguished from signals caused by light emitted by another light source of another wavelength of the plurality of light sources, by the frequency analyzer.

16. The system of claim 15, wherein the dichroic fold mirror folds light emitted by each light source of the plurality of light sources into one output of light directed to a target.

17. The system of claim 16, wherein the light detector comprises an annular detector.

18. The system of claim 17, wherein the light detector comprises an array of concentric annular detectors.

19. The system of claim 17, wherein the target is a core stream of a flow stream channel of a cytometer.

20. The system of claim 19, wherein the light detector comprises a FALS detector.

21. The system of claim 20, wherein the light detector comprises a SALS detector.

22. The system of claim 21, wherein the light detector comprises a counting detector

23. The system of claim 22, further comprising:

a second light detector proximate to the flow stream channel; and

wherein a portion of the one output of light is directed to another part of the flow stream channel.

24. The system of claim 23, wherein signals from the first and second light detectors may have velocity information about the core stream.

25. A method for identifying components of a detected light beam having different wavelengths, comprising:

modulating with a first frequency a first light having a first wavelength;

modulating with another frequency at least another light having another wavelength;

combining the light having the first wavelength with the at least another light with the another wavelength into a light beam;

detecting the light beam with a detector that converts the detected light into an electrical signal; and

analyzing the electrical signal into signals representing light of the first wavelength and signals representing the at least another light having another wavelength.

26. The method of claim 25, further comprising directing the light beam at a target.

27. The method of claim 26, wherein the target is a core stream of a cytometer flow stream channel.

28. The method of claim 26, wherein the detector detects light scattered by the target.

29. The method of claim 28, wherein the detector is an annular detector.

30. The system of claim 29, wherein the detector has an array of concentric annular detectors.

31. The method of claim 28, wherein the detector comprises an array of individual detectors for

determining amounts of light at different angular intervals of direction of the light.

32. Means for identifying components of a light according to wavelength from an electrical signal representing the light, comprising:

means for providing a first light having a first wavelength;

means for providing at least another light having another wavelength;

means for modulating the first light with a first frequency;

means for modulating the at least another light with another frequency;

means for combining the first light and the at least another light into a single light;

means for converting the single light into an electrical signal; and

means for analyzing the electrical signal into a first signal that represents the first light and at least another signal that represents the at least another light, according to the first frequency and another frequency, respectively.

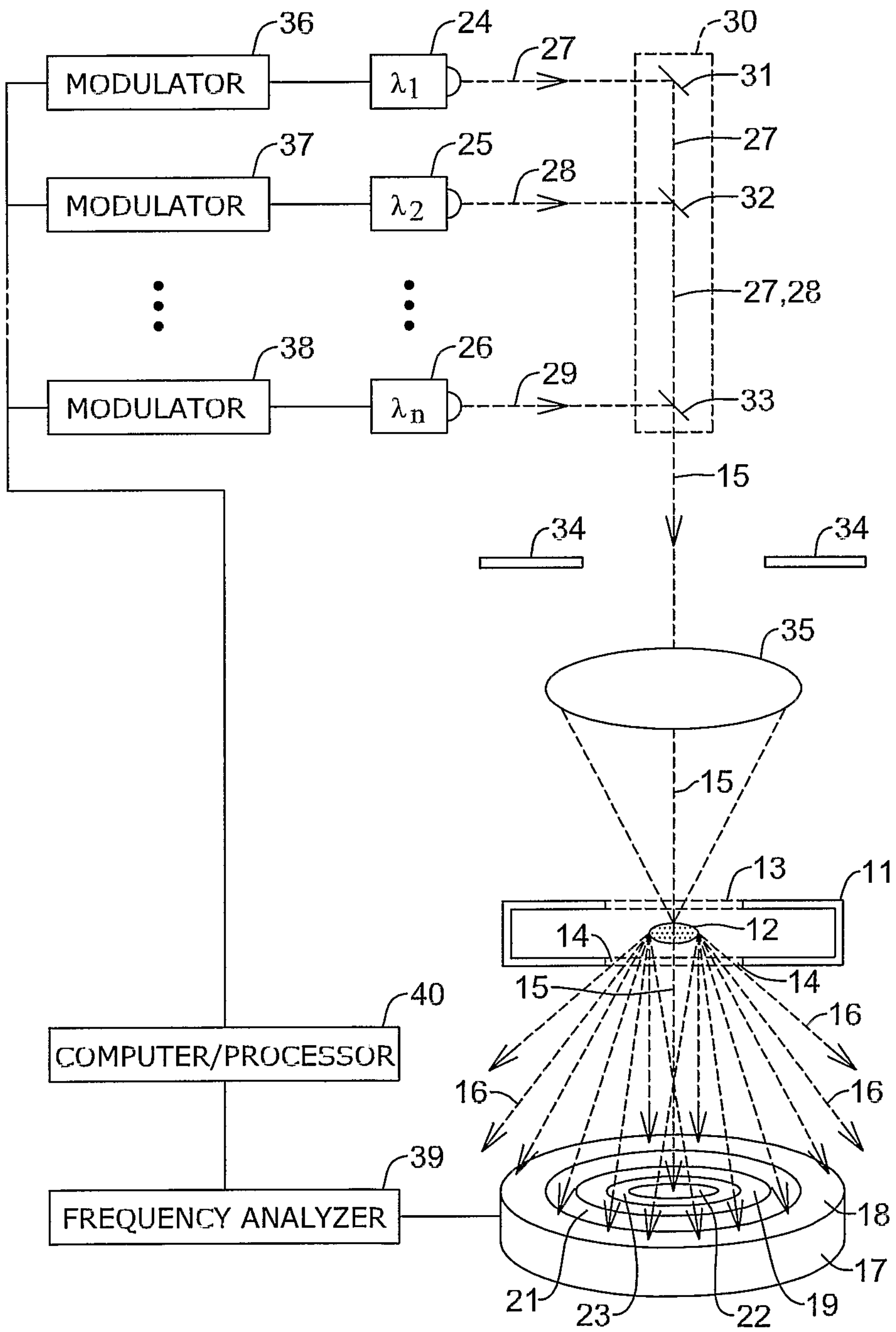
33. The means of claim 32, further comprising a means for directing the single light to a target.

34. The means of claim 33, wherein the target is a core stream of a flow stream channel of a cytometer.

35. The means of claim 34, wherein the single light is scattered light.

36. The means of claim 35, wherein the means for converting the single light into an electrical signal further comprises detecting light scattered at various angles and converting the light into electrical signals representing the light of the various angles.

37. The means of claim 35, wherein the means for segregating the electrical signal is a frequency analyzer.



MODULATOR
MODULATOR
MODULATOR
λ1
λ2
λn
COMPUTER/PROCESSOR
FREQUENCY ANALYZER
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Figure 1

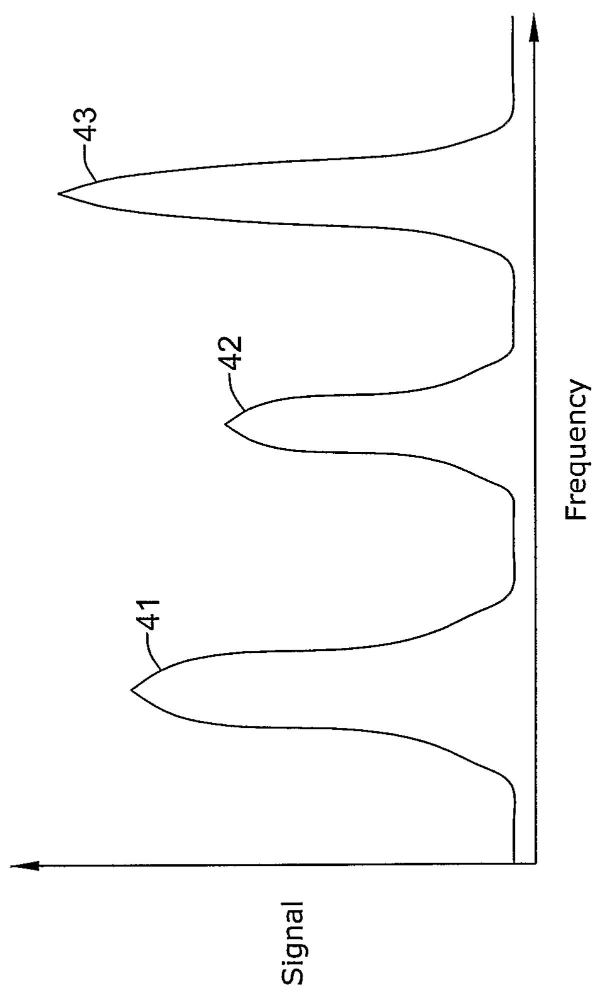

Figure 2

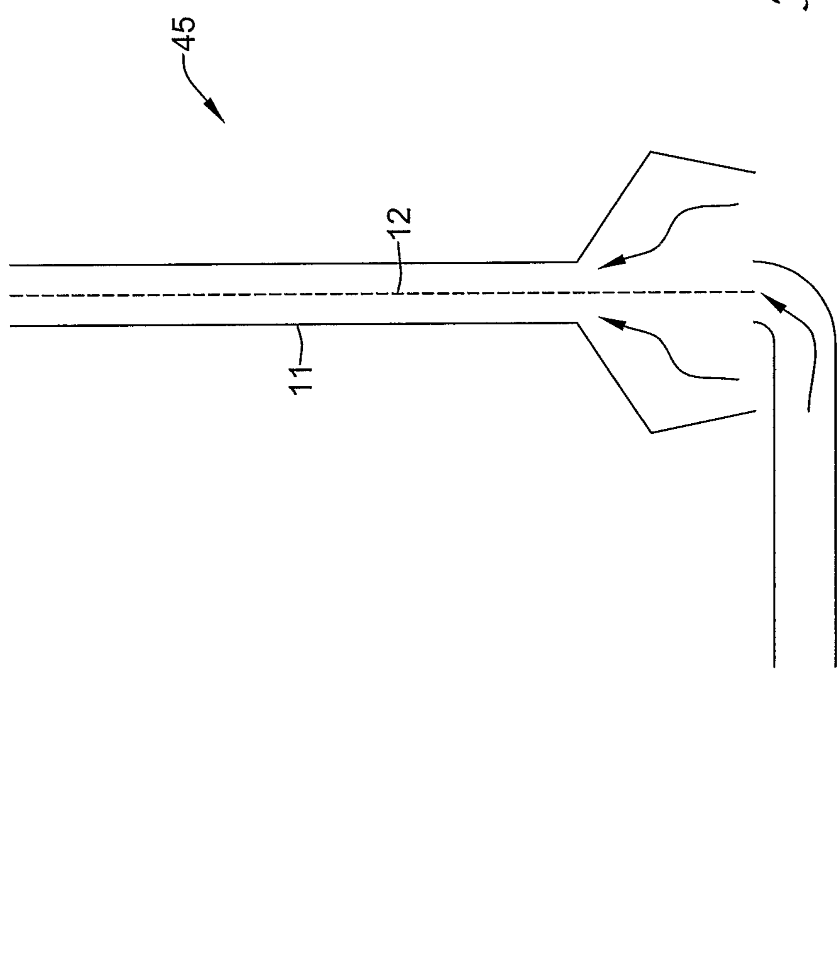

Figure 3