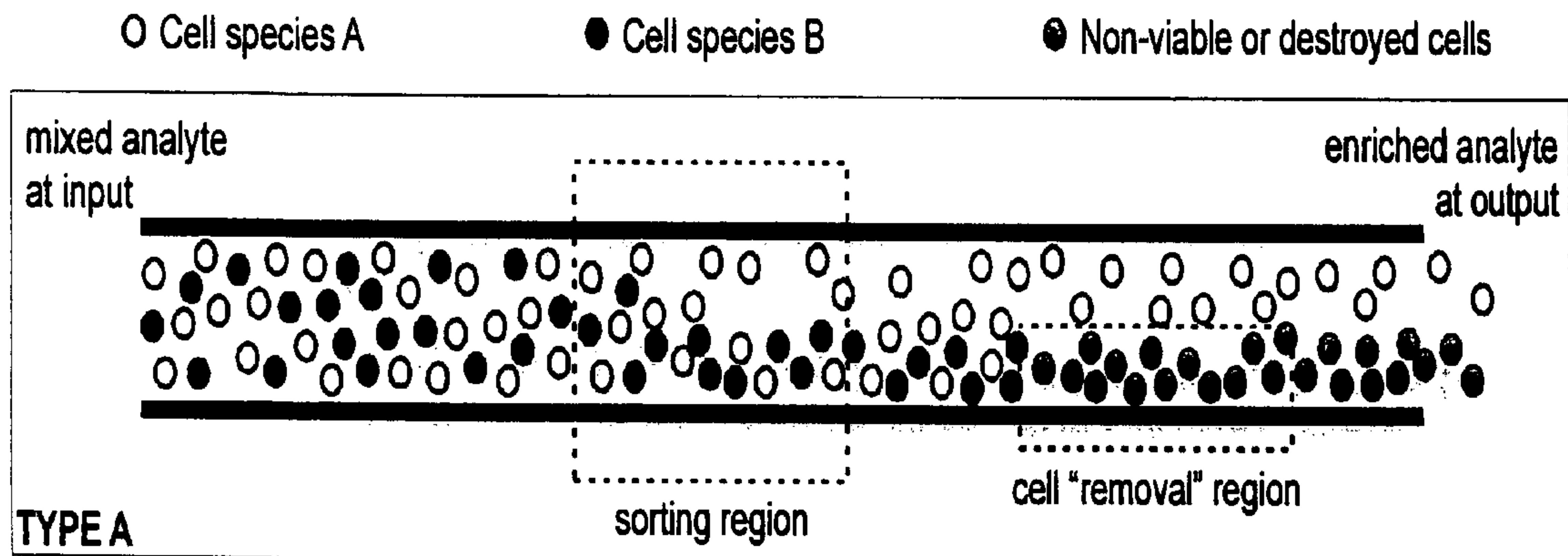




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(72) **Inventeurs/Inventors:**
MACDONALD, MICHAEL, GB;
DHOLAKIA, KISHAN, GB;
ANDREEV, IGOR, GB
(73) **Propriétaire/Owner:**
THE UNIVERSITY COURT OF THE UNIVERSITY OF ST
ANDREWS, GB
(74) **Agent:** MARKS & CLERK

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A method for sorting particles, in particular cells A and B. The method uses a single channel with only one input and only one output. A particle mix A and B in a fluid is introduced into the channel and particles within the channel are sorted.

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Fife KY16 9SS (GB). **ANDREEV, Igor** [GB/GB]; School of Physics & Astronomy, University of St Andrews, North Haugh, Fife KY16 9SS (GB).

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(74) **Agents: NAISMITH, Robert, Stewart** et al.; Marks & Clerk, 19 Royal Exchange Square, Glasgow G1 3AE (GB).

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(71) **Applicant** (*for all designated States except US*): **THE UNIVERSITY COURT OF THE UNIVERSITY OF ST ANDREWS** [GB/GB]; College Gate, North Street, St Andrews (GB).

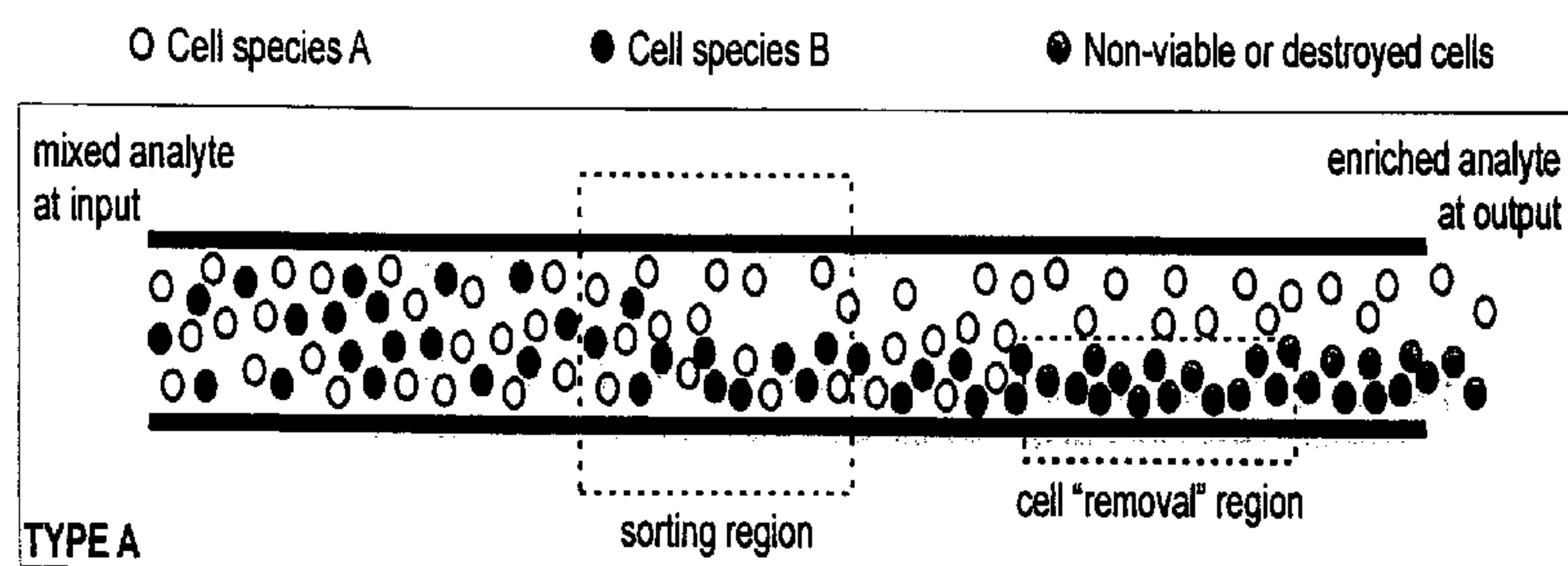
(72) Inventors; and

(75) **Inventors/Applicants** (*for US only*): **MACDONALD, Michael** [GB/GB]; School of Physics & Astronomy, University of St Andrews, North Haugh, Fife KY16 9SS (GB). **DHOLAKIA, Kishan** [GB/GB]; School of Physics & Astronomy, University of St Andrews, North Haugh,

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(54) Title: OPTICAL SORTING



(57) **Abstract:** A method for sorting particles, in particular cells A and B. The method uses a single channel with only one input and only one output. A particle mix A and B in a fluid is introduced into the channel and particles within the channel are sorted.

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OPTICAL SORTING

The present invention relates to optical sorting of particles, and in particular cells.

5 BACKGROUND OF THE INVENTION

Many particle sorting or separation schemes exist, ranging from gel-electrophoresis, capillary electrophoresis, and analytical centrifuging to novel, entropic barriers. Examples of these are described by J. Han, H. G. Craighead, Science 288, 1026-1029 (May 12, 2000) and D. Nykypanchuk, H. H. Strey, D. A. Hoagland, Science 297, 987-10 990 (Aug 9, 2002). The majority of these known techniques separate a polydisperse mixture in a flowing fluid into bands containing particles that travel at different velocities along the direction of flow. This typically leads to batch processing. In electrophoresis a gel is used to obtain a size-dependent mobility. Recovery of fractions is achieved through post-processing of the gel. However, despite its 15 widespread use and effectiveness this methodology is slow and importantly, due to limited pore sizes, has difficulty in separating objects at the microscopic size level, for example cells, chromosomes and colloidal matter.

Lithographically fabricated two-dimensional, asymmetric artificial gels are also used. 20 Examples of these are described by D. Ertas, Physical Review Letters 80, 1548-1551 (Feb 16, 1998); T. A. I Duke, R. H. Austin, Physical Review Letters 80, 1552-1555 (Feb 16, 1998) and C. F. Chou et al., Biophysical Journal 83, 2170- 2179 (Oct, 2002). These gels yield separation transverse to the direction of flow. Because of this, they can be operated in a continuous fashion, with various fractions taken up by separate 25 collection channels. However, sorting based on diffusion is impractically slow at the microscopic scale.

In recent years there has been growth in the exploration of particle motion on optical landscapes. An example of this is described in the article "Kinetically Locked-in 30 Colloidal Transport in an Array of Optical Tweezers" by P. T. Korda et al, Physical Review Letters 89, Number 12, Art. No. 128301 (16 Sep, 2002). In this case, a monolayer of colloidal spheres is allowed to flow through an array of discrete optical traps. By varying the orientation of the array of traps, the direction of flow of the spheres can be varied. Because of this, it has been suggested that the lattice could be

used to continuously fractionate mesoscopic particles. However, because of the use of a lattice of localized discrete traps, the observed kinetically locked-in channelling along low-index lattice vectors is intrinsically limited to small-angle deflections. In practice, this limits the practicality of the lattice for use in fractionation.

5

PCT/GB2004/001993 describes yet another optical fractionation scheme. In this, three-dimensional optical lattices are used for sorting and fractionation of biological and colloidal material in a microfluidic flow. Different particles follow different trajectories across the landscape and consequently exit at different points. The selectivity and basis of this form of sorting is the affinity of a given particles to the features of the optical landscape. This is also described by M. MacDonald, G. Spalding and K. Dholakia, in Nature 426, 421 (2003), and by A. M. Lacasta, *et al.*, in Physical Review Letters (2005), 94, 188902. Even in the absence of fluid flow periodic optical patterns may be used for sorting, see L. Paterson, *et al.*, Applied Physics Letters (2005), 87, 123901.

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One of the main advantages of using optically defined microfluidic sorting is that the requirements on the physical microfluidics can be kept to a minimum. Nevertheless, in some circumstances there is a need for an even simpler arrangement.

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SUMMARY OF THE INVENTION

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According to the present invention, there is provided a sorting method that uses a single microfluidic channel or other suitable conduit such as a microcapillary having an elongate channel with only one exit in the fluid flow direction, the method involving introducing a particle mix in a fluid into the channel, and optically sorting particles within the channel. Once the particles are sorted, they can then be treated further whilst still within the channel.

30

By providing a very simple channel arrangement and optically sorting particles within that channel, no physical separation of sorted particles into separate channels is required.

Sorting the particles may involve physically separating them into different regions of the channel or subjecting the particles to an optical potential that only one type is

sensitive to. In the latter case, the particle mix may be subjected to an optical potential that preferentially damages or de-activates one type of particle, so that the output is a sample that is enriched with one or more other types of particle. Where cells are being sorted, the optical potential may be chosen to preferentially kill or damage one type of cell. For example the method may be for sorting white and red cells and may involve using an optical field to cause the red cells to flip, that is rotate by 90 degrees and align with the light field, thereby causing physical damage to those cells, for example to the cell membrane. The optical field may comprise an optical funnel. One or more lines of light may be used to define the optical funnel.

The method may involve processing, for example treating and/or sampling and/or measuring a characteristic of, the sorted particles whilst still within the channel. The sorted particles that are processed may be all of one type or may be a mix of particle types.

In one embodiment, a laser is used to kill cells in part of the flow after sorting, so that only the desired cells leave the channel alive or active. In this way, a single channel can be used with only one input and one output.

Alternatively, instead of killing unwanted cells, selected cells can undergo a second optical process whilst still within the sorting channel. The second optical process could be for example optoporation as described in WO2006/059084.

Additionally or alternatively, the second optical process may be some form of spectroscopy, such as raman spectroscopy, as described in our co-pending patent application GB 0611289.0.

The optical sorting may be done using an optical landscape or pattern that is defined by an acousto-optic device, as described in our co-pending patent application GB 0618606.8.

According to another aspect of the invention, there is provided a system for sorting particles, in particular cells, comprising a single channel with only one output and means for sorting particles within that channel.

- 5 Preferably, the single channel has a single input and the particle mix is introduced via that input.

The channel may be a microfluidic channel and particles are preferably introduced in a fluid flow. Preferably the channel is a micro-capillary.

10

Preferably, the means for sorting comprise means for sorting the particles optically. The means for optically sorting the particles may comprise an acousto-optic device.

- 15 Means may be provided for treating and/or sampling and/or measuring a characteristic of the sorted particles, whilst still within the channel. The means for treating may comprise means for killing or de-activating particles in at least part of the channel and/or means for porating particles in at least a part of the channel. The means for measuring may comprise means for measuring a spectra, for example a raman spectra.

- 20 According to one aspect of the invention there is provided a method for sorting cells comprising:

- providing a single channel with only one output;
- introducing a mix of cell types in a fluid into the channel;
- in a sorting region in the channel, optically deflecting one of the cell types to
- 25 cause the deflected cells to flow into or away from a removal region that extends only partially across a width of the channel downstream from the channel sorting region; and
- killing or de-activating by means of a laser cells that flow into the removal region, thereby providing an enriched sample at the channel output.

- 30 According to a further aspect of the invention there is provided a system for sorting cells comprising:

- a single channel with only one output;

4a

a sorting region in the channel for optically deflecting one of the cell types of a mix of cell types to flow into or away from a removal region that extends only partially across a width of the channel downstream from the channel sorting region; and

5 a laser for killing or de-activating cells that flow into the removal region, thereby providing an enriched sample at the channel output.

BRIEF DESCRIPTION OF THE DRAWINGS

Various aspects of the invention will now be described by way of example only and with reference to the accompanying drawings, of which:

10 Figure 1 is cross-section through a micro-fluidic channel with a cell sorting region and a cell removal region;

Figure 2 shows a variation on the arrangement of Figure 1, and

Figure 3 shows an alternative micro-fluidic cell sorting arrangement.

15 SPECIFIC DESCRIPTION OF THE DRAWINGS

Figures 1 and 2 show a single microfluidic channel for sorting, for example cells. The channel is cylindrical with a constant cross section, its ends defining an input and an output. The channel has a sorting region in series with a cell-processing region, for example an optical cell removal region. An analyte of interest is injected into one end
20 of the micro-channel, flows through the channel and into the sorting region, where sorting is done using any suitable means, preferably optical means. Techniques for

doing this are known in the art and so will not be described in detail. The optical sorting is done using an optical arrangement that is effective in a localised region of the channel, and not along its full length.

5 Once the cells are sorted, they move downstream in the fluid flow to the cell removal region. This region extends only partially over the flow path, so that some cells enter it, but others do not. Within this region cells are removed by a laser that makes them either non-viable or destroys them. To achieve this, a portion, typically half, of the channel, as measured across its width, is illuminated with a laser at a wavelength and
10 average power/peak power that will kill or damage any cell. The material that is then taken from the other end of the conduit contains an enriched flow of cells, as only the cells that have not passed through the removal region are viable.

Figure 1 shows how a pure flow of cells can be created from a mix of cells A and B,
15 but with the loss of about half of the desired cell species. In this case, the cell removal region extends over a lower portion of the channel and the optical potential/landscape in the optical sorting region is arranged so that the B cells are deflected towards the lower part of the channel, but the A cells are substantially unaffected and remain distributed throughout the channel. When the particle mix flows into the cell removal
20 region, the A cells are present in the upper channel region, but a mixture of the A and B cells is present in the lower region of the channel. Since the cell removal region extends partially over the lower part of the channel, the A cells in the upper region pass through unaffected, whereas cells in the A and B mix are damaged or killed or made otherwise biologically inactive. Therefore, at the channel output only A cells
25 are active, thereby providing an enriched analyte.

Figure 2 shows a variation on Figure 1, in which a sample mix of A and B cells can be sorted and processed to provide an enrichment of approximately 50%, whilst at the same time ensuring that no cells of the desired species are destroyed. In this case, the
30 cell removal region extends over the upper part of the channel. As before, the optical potential/landscape in the optical sorting region is arranged so that the B cells are deflected towards the bottom of the channel as the fluid flows through the cell-sorting region, but the A cells are substantially unaffected and remain distributed throughout the channel. Hence, when the A and B cell mix flows into the cell removal region, A

cells are present in the upper channel region, but a mixture of A and B cells is present in the lower region of the channel. In this case, because the cell removal region extends partially over the upper region of the channel, only A cells are exposed to the removal radiation. Hence, as the A and B cell mix flows through the removal region the A cells in the upper region are rendered biologically inactive, whereas cells in the A and B mix in the lower region are unaffected.

Whilst the arrangement of Figure 2 results in a mix of live cells at the output, this mix will have more B cells than A cells. Re-circulating the fluid will lead to higher levels of enrichment. This could be achieved simply by switching of the sorting and cell removal regions, reversing the flow and re-starting process. This approach might be attractive when trying to sort out cell species with a very low population compared to other cell types in the analyte.

Whether the arrangements of Figure 1 or Figure 2 are used depends upon the ratio of cell species entering the channel, the level of enrichment required by the user, and whether or not it is acceptable to lose 50% of the desired cells.

As an alternative to causing damage, sorted cells could be processed. For example, in the example of Figure 1 the A cells in the upper part of the channel could be targeted by a second laser and porated, such that either a chemo or gene agent within the medium can be transfected into them. As an example, transfection could be used to test for antibiotic resistance or for express of green fluorescent protein. In a preferred embodiment the porating laser output is in the form of a Bessel beam but may also be achieved with a gaussian beam. As described in WO2006/059084, this can provide significant technical advantages. Hence, in the output there will be a select population of cells that have been treated. Another option is to obtain Raman data from the sorted cells. This can be done using the techniques described elsewhere.

Figure 3 shows another optical landscape for sorting cells in a micro-capillary. The optical landscape works by subjecting the cells to an optical field that only one type is sensitive to. In this case, the landscape is an optical funnel that focuses down a broad flow of particles into a single or dual file flow of particles. The cell mix is subjected

to an optical field that damages or otherwise de-activates one type of cell, so that it ceases or is unable to fulfil its biological function. The result is that the output is a sample that is enriched with one or more other types of cell.

Advantageously, the arrangement of Figure 3 can be used for sorting red cells from
5 white cells. Red cells are bi-concave discs and white cells are generally spherical. The optical landscape used to sort these is in two dimensions and typically is funnel shaped, with the funnel narrowing in the direction of fluid flow. A fluid containing a mixture of red and white cells is introduced into the micro-capillary and caused to flow towards the narrow end of the funnel. The flow of white cells is focussed down
10 by the funnel but otherwise passes through unaffected. In contrast, the red cells flip to align with the narrow output of the optical funnel and become damaged or lysed due mechanical stresses induced in the violent flipping process, so that only active white cells make it through. In this way, the output is enriched with white cells.

15 A skilled person will appreciate that variations of the disclosed arrangements are possible without departing from the invention. Accordingly the above description of the specific embodiment is made by way of example only and not for the purposes of limitation. It will be clear to the skilled person that minor modifications may be made without significant changes to the operation described.

20

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A method for sorting cells comprising:
providing a single channel with only one output;
introducing a mix of cell types in a fluid into the channel;
in a sorting region in the channel, optically deflecting one of the cell types to cause the deflected cells to flow into or away from a removal region that extends only partially across a width of the channel downstream from the channel sorting region; and
killing or de-activating, by means of a laser, cells that flow into the removal region, thereby providing an enriched sample at the channel output.
2. A method as claimed in claim 1 wherein the single channel has a single input and the mix of cell types is introduced via that input.
3. A method as claimed in claim 1 wherein the channel is a microfluidic channel and the mix of cell types is introduced in a fluid flow.
4. A method as claimed in any one of claims 1 to 3 comprising treating and/or sampling and/or measuring a characteristic of the sorted cells whilst still within the channel.
5. A method as claimed in claim 4 comprising porating cells in at least a part of the channel.
6. A method as claimed in claim 5 comprising introducing material into the porated cells.
7. A method as claimed in claim 4 comprising making a spectroscopic measurement of the sorted sample.

8. A method as claimed in claim 7 wherein the spectroscopic measurement is a Raman spectra.
9. A method as claimed in any one of claims 4 to 8 wherein treating and/or sampling and/or measuring of the sorted cells is done in a region that extends partially across the channel.
10. A method as claimed in any one of the claims 1 to 9 wherein the single channel is a micro-capillary.
11. A method as claimed in any one of claims 1 to 10 for sorting white and red blood cells comprising using an optical field to causing the red cells to flip.
12. A method as claimed in claim 11 wherein the optical field comprises an optical funnel.
13. A system for sorting cells comprising:
 - a single channel with only one output;
 - a sorting region in the channel for optically deflecting one of the cell types of a mix of cell types to flow into or away from a removal region that extends only partially across a width of the channel downstream from the channel sorting region; and
 - a laser for killing or de-activating cells that flow into the removal region, thereby providing an enriched sample at the channel output.
14. A system as claimed in claim 13 wherein the single channel has a single input and the cell mix is introduced via that input.
15. A system as claimed in claim 13 or 14 wherein the channel is a microfluidic channel and cells are introduced in a fluid flow.

16. A system as claimed in any one of claims 13 to 15 comprising means for treating and/or sampling and/or measuring a characteristic of the sorted cells, whilst still within the channel.

17. A system as claimed in claim 16 comprising means for porating cells in at least a part of the channel.

18. A system as claimed in claim 17 comprising means for introducing material into the porated cells.

19. A system as claimed in any one of claims 16 to 18 comprising means for measuring a characteristic spectra.

20. A system as claimed in claim 19 comprising means for measuring a raman spectra.

21. A system as claimed in any one of claims 13 to 20 wherein the single channel is a micro-capillary.

22. A system as claimed in any one of claims 13 to 21 for sorting white and red blood cells comprising means for forming an optical field to cause the red cells to flip.

23. A system as claimed in claim 22 wherein the optical field comprises an optical funnel.

24. A system for sorting cells comprising:
a single channel;

a sorting region within the channel for deflecting one of the cell types of a mix of cell types to flow into or away from a cell treatment or sampling or measurement region that extends only partially across a width of the channel downstream from the channel sorting region; and

means for treating or sampling or measuring cells within the cell treatment or sampling or measurement region.

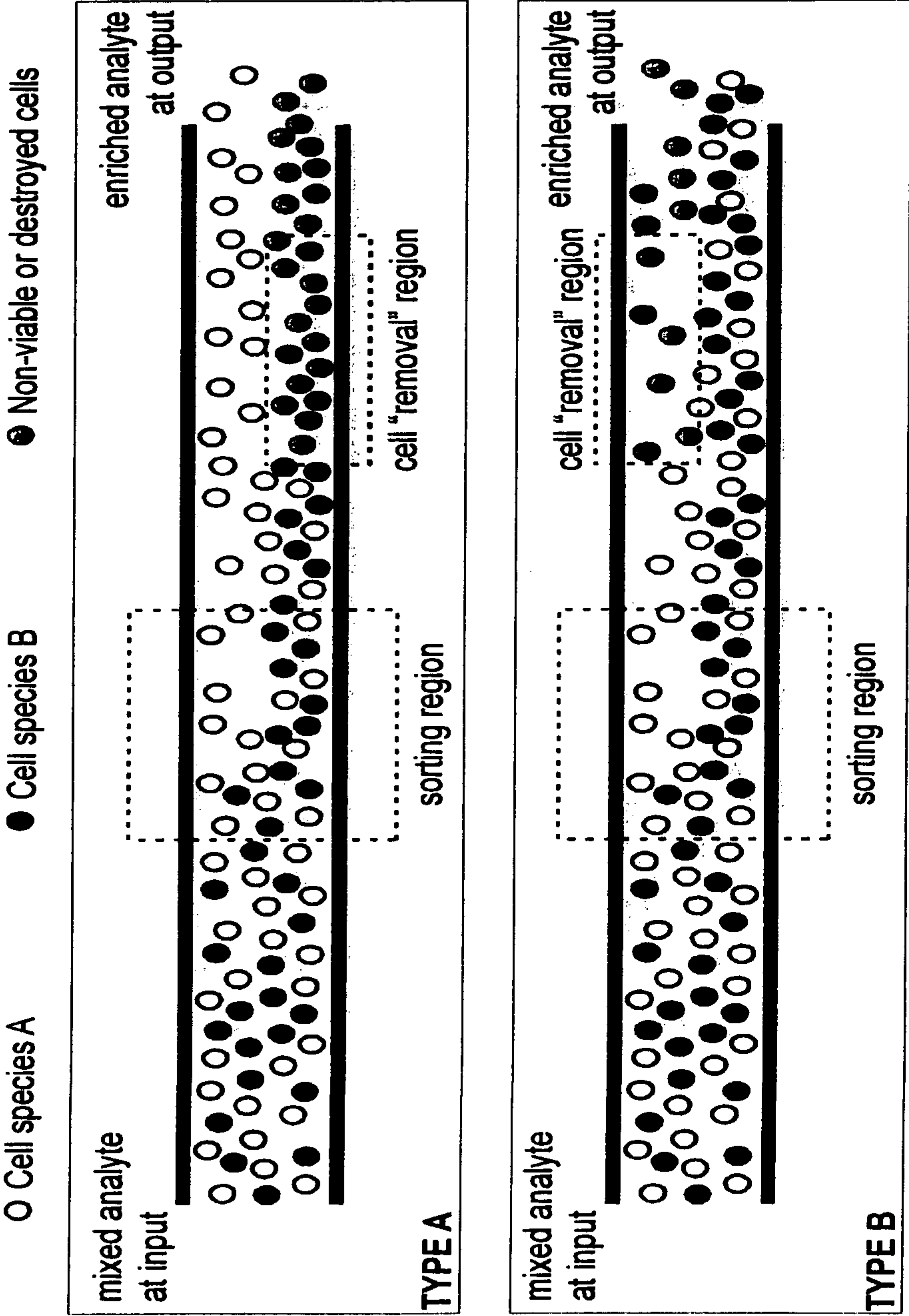


Figure 1

Figure 2

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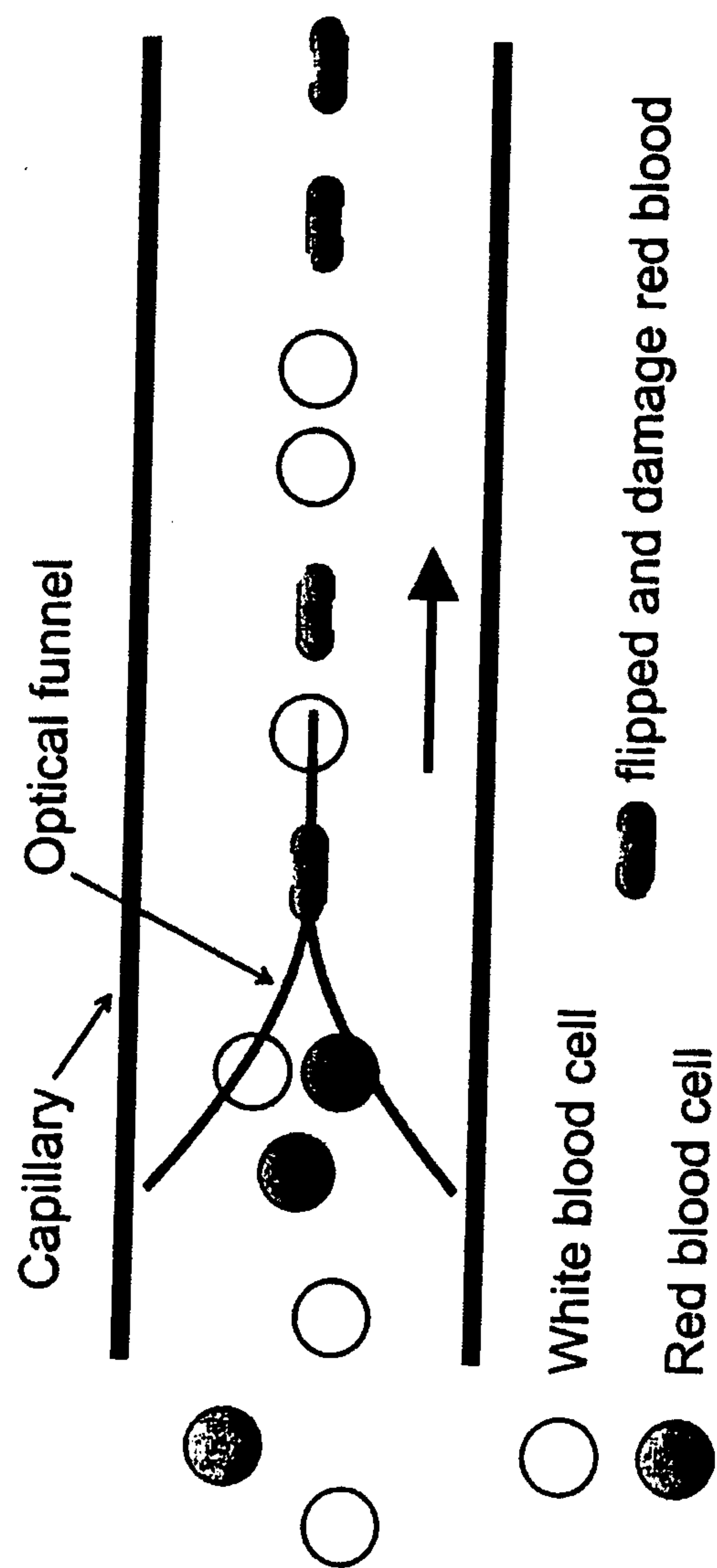


Figure 3

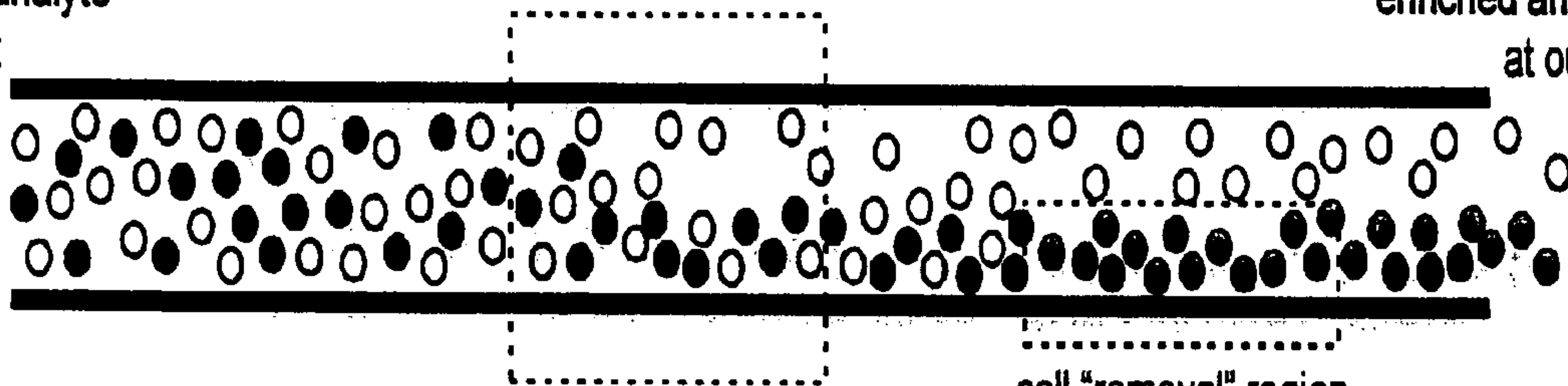
○ Cell species A

● Cell species B

◐ Non-viable or destroyed cells

mixed analyte
at input

enriched analyte
at output



sorting region

cell "removal" region

TYPE A