Natural plasmid pSD89 (Cm^r) was extracted from clinical isolate of *Salmonella derby* K89 [2] which was resistant to several antibiotics. Strains of *S. derby* K89 and K82 (cured of plasmids) manifest the same resistance to UV-light but original strain is more sensitive to ionizing radiation. Several plasmids with molecular mass 2 - 60 Md were found by means of electrophoresis in agarose gel. Cm^r transformants which were more resistant to gamma-rays and UV-light were selected after transformation of *S. derby* K82. Plasmid pSD89 (Cm^r) was modified in *E. coli* K-12 strain Z805 (r^- m^+). Varios repair-deficient *E. coli* strains were transformed with pSD89 (Cm^r) and their radiation-sensitivities were evaluated. Plasmid pSD89 (Cm^r) increases radiation-resistance of *E. coli* wild-type and compensates defect in great extent in *polA*, *recA* mutants but not in *uvrA* and *umuC* mutants (Table).

It is likely that plasmid pSD89 (Cm^r) from *S. derby* K89 is carrying locus determining product which does take part in repair but is not identical to known MucA and MucB proteins.

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KARYOTYPING OF INDIVIDUAL CELLS WITH FLOW CYTOMETRY

S.I.Stepanov, V.N.Konyshev, L.V.Kotlovanova and A.P.Roganov

As the study of metaphase chromosomes with flow cytometry presupposes mixing all chromosomes from many cells before analysis, important information is lost. To overcome this limitation we have developed a novel cell-oriented flow cytometric method for chromosome analysis [1-3].

A flow cytometer supplied with special Device for disruption of metaphase chromosomes is the heart of the method (Fig.1). Cells with stained chromosomes are pressed into the Disruption Device and then converted in batches of chromosomes. Chromosome fluorescence and time intervals between fluorescence pulses are registered. There are large time intervals between neighboring batches because a sparse cell suspension is used and, in turn, there are small time intervals inside batches. Taking into account of time intervals it is possible to identify individual cell flow karyotypes (Fig.2 and 3).

The novel method possesses the following features:

- Unique ability to obtain individual cell flow karyotypes (Fig.3B and 3C);
- High technologic principle based on ordinary flow cytometer modified with one accessory - Disruption Device;
- Small number of cells needed for analysis (some thousands);
- Automatical acquisition and analysis of data;
- High rate of acquisition, 100-200 cells/min;

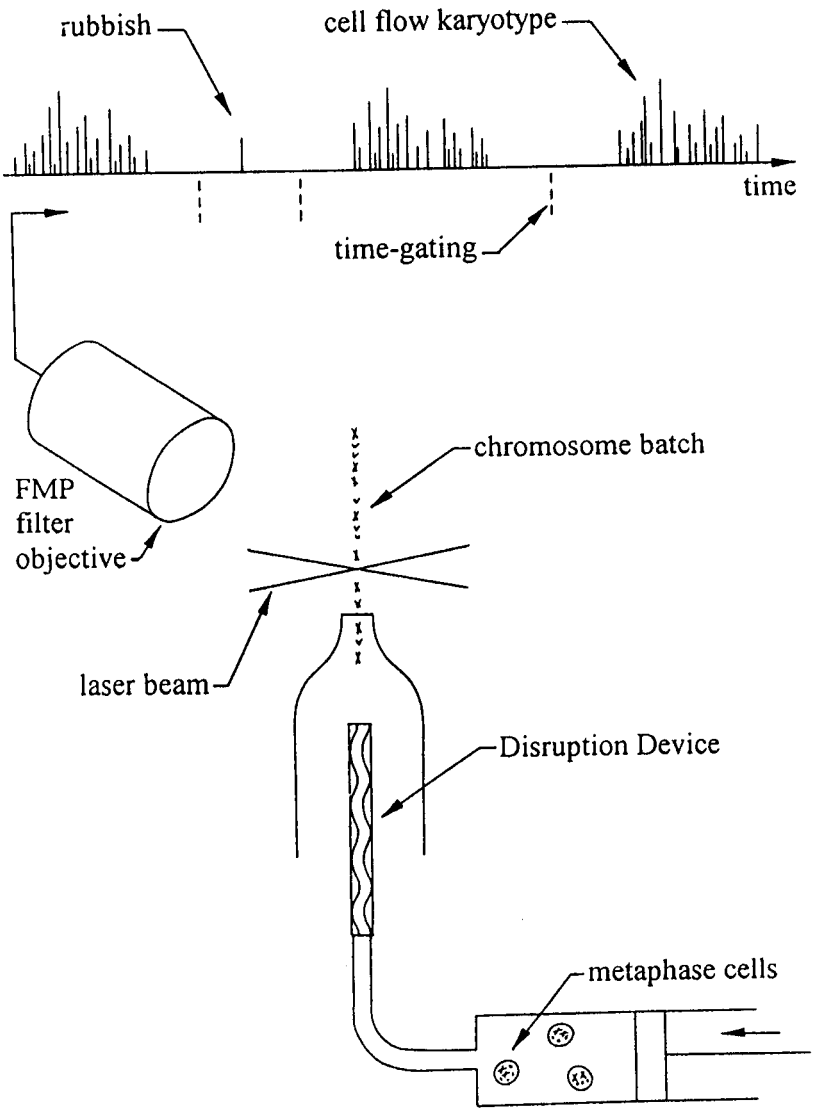

Fig.1. The scheme of single cell flow cytometric chromosome analysis. An ordinary flow cytometer supplied with Disruption Device is used in the method. The suspension of metaphase cells with stained chromosomes flows from the syringe into the Disruption Device installed in the flow chamber. The Disruption Device converts a cell into a batch of chromosomes. The electronics measure amplitudes of fluorescence pulses and time intervals between pulses and yield listmode data (on the top). As a sparse cell suspension (approximately 2000 cells/ml) is used, the time intervals between batches are significantly larger than those within a batch. This allows use of time gating to obtain individual cell flow karyotypes

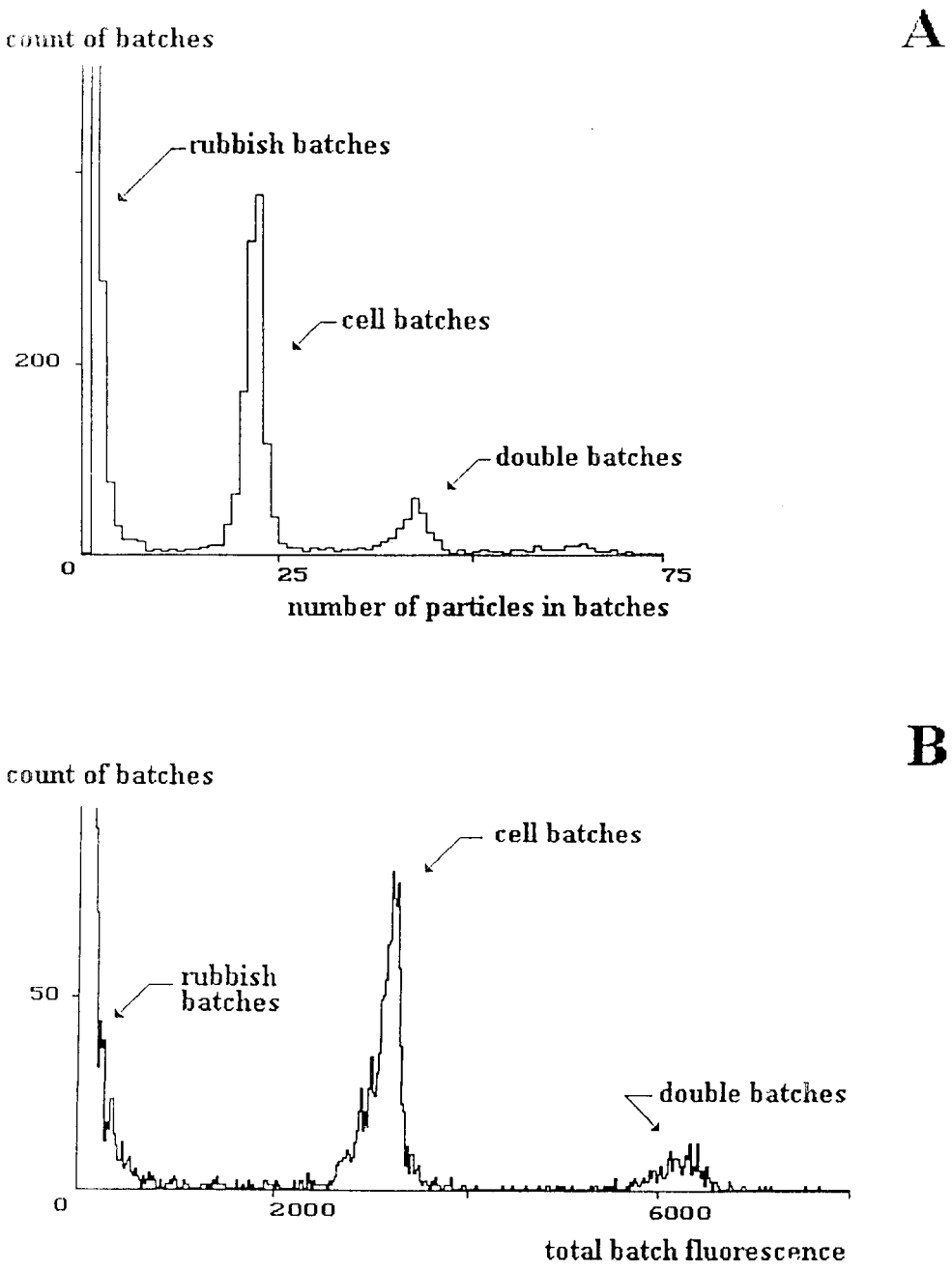

Fig.2. All the batches which comprise the listmode data are presented as the number of particles in batches (A) and as total batch fluorescence (B). Total batch fluorescence is the sum of fluorescence amplitudes in a batch. Batches of three types are seen: rubbish; cell batches consisting of metaphase chromosomes; and double cell batches

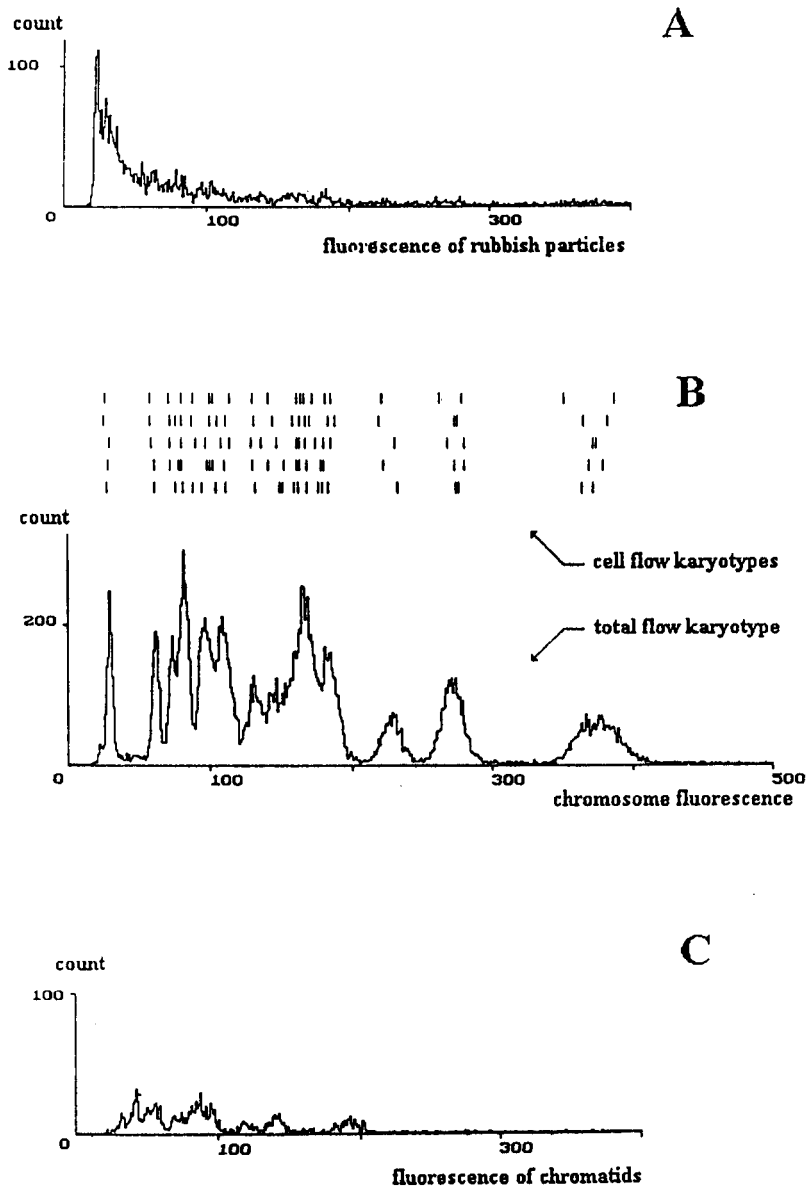

Fig.3. Fluorescence of particles in the batches of some types. **A**: Rubbish: the number of batch particles is 1 to 5. The rubbish fluorescence appears as smooth continuum. **B**: Chromosomes of metaphase cells: the number of batch particles (chromosomes) is 15 to 25, total batch fluorescence is 2400-3400 (diploid DNA content). The examples of cell flow karyotypes are presented. Approximately 1000 cell karyotypes were registered during 10 min. In a cell flow karyotype any chromosome changes can be easily revealed. These are the absence of a chromosome in an expected position (underrepresentation), overrepresentation, or the appearance of a chromosome in a unusual position. Analisis of each cell karyotype determines whether the cell is normal or aberrant. **C**: Chromatids were selected from batches which have the number of particles 35 - 45 and total batch fluorescence of 2400 - 3400 (diploid DNA content). The measured incidence of cells with chromatids is 1%

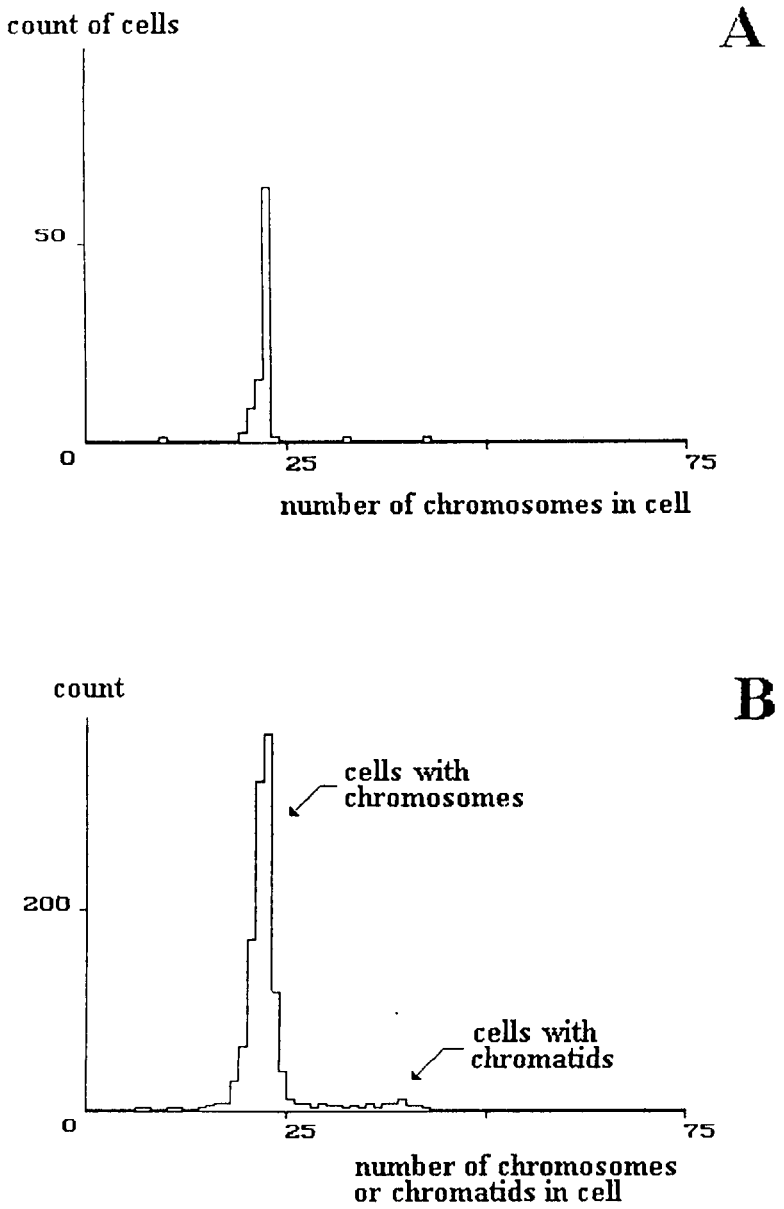

Fig.4. The number of chromosomes (chromatids) in cells counted by the novel method. To generate this plot, the batches with total batch fluorescence of 2400-3400 (diploid DNA content) were taken. A few cells with chromatids are seen on the right

— Precision of analysis which ensures detecting a change of a single chromosome in the cell flow karyotype.

We suppose, the method will be effective in studying chromosomal aberrations and classification of cell karyotypes, and in other tasks of biology and medicine.

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DISCRETE PREPARATIVE ELECTROFRACTIONATION OF COMPLEX PROTEIN MIXTURE

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The purification of homogeneous protein is the main task in its study. The excellent resolution of SDS-PAGE in analysis of proteins stimulated the creation of various preparative devices in which bands emerging from the bottom of electrophoresis gels are swept away to fraction collector [1-2]. Though the efficiency of this technique was reported as being high, some problems still exist. Among them, strong dilution of protein zones by the eluting buffer, complexity of elution chamber and high cost of commercial devices. The described method and apparatus allow to overcome all these problems. The essence of the method is that separation is carried out in a gel cylinder and fractionation is accomplished by new patented manner which is distinguished by overturning of the gel cylinder and up-side discrete protein fractions collection in maximum concentration by means of pipette. It's necessary to emphasize the concentrating effect of this method which allows to find a faint residual enzyme activity after its SDS-denaturation. The efficiency of this technique was demonstrated by the purification of one of protein kinases and error-correcting 3'-5'exonuclease from rat liver.

The schematic view of the electrophoresis system is shown in Fig.1. The separation is carried out in a gel cylinder according to Laemmli (3). The glass tube with a gel is fixed in the apparatus, standard electrophoresis buffer is poured into the both reservoirs (B) and sample is loaded onto the gel. Electrophoresis is carried out until the electrophoresis front (bromphenol blue) reachs the lower edge of the resolving gel (Position 1, Fig 1). Power is turned off and instrument is overturned. Then power is switched on again and electrophoresis is going on. Now protein zones are migrating from bottom to top (F) and gathering in section E where they are being drawn with a pipette (Position 2, Fig.1). For this case a buffer level (C) is lowered (D) and section E is separated from the main reservoir. This is done by means of unit G.

Electrofractionation results showing a distribution of protein zones in eluted fractions are represented in Fig.2. The eluted fractions were analyzed by standard SDS-PAGE [3] and stained by silver.