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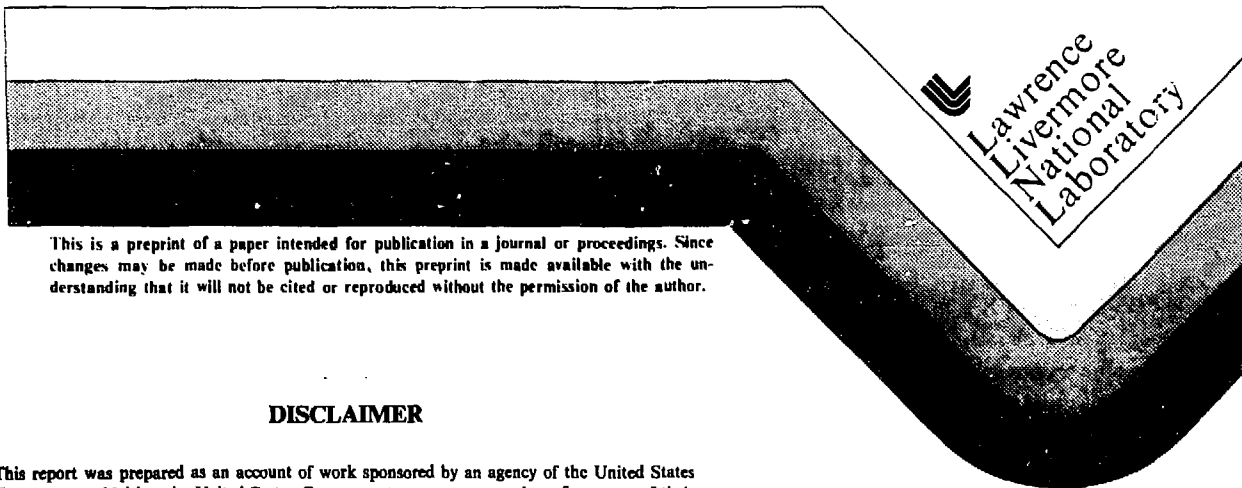
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# A COMMERCIAL BACTERIAL COLONY COUNTER FOR SEMIAUTOMATIC TRACK COUNTING

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## ABSTRACT

The information one must obtain from a solid state track detector depends on the specific application. The most common information need is the measurement of track density. The number of tracks per unit area is commonly used in neutron and alpha dosimetry, for example, to determine radiation dose.

In recent years, a class of semi-automatic counting systems has become available for under \$15,000. These systems, usually developed for bacterial colony counting, are capable of measuring track density. The basic instrument is designed to count relatively large objects (>100 $\mu$ m) with low magnification using an illuminated stage. However, track counting can be done with an accessory television camera coupled to an optical microscope. Tracks from electrochemical etching can be counted easily with objectives as low as 2X.

## KEYWORDS

Track detectors; optical counting; track density; track size; semi-automatic counting.

## INTRODUCTION

Experimenters and dosimetrists who use solid state nuclear track detectors require information that ranges in complexity from simple areal track density to details about individual tracks (length, cone angle, orientation, diameter, etc.). Optical techniques are usually used to obtain this information. Most generally, tedious manual counting and measurements using an optical microscope are necessary. Some laboratories have the technical or financial resources to either purchase and adapt expensive commercial pattern recognition systems (Dutrannois and Sullivan, 1979; Benton and others, 1981). Others (Palfalvi, Eordogh and Vero, 1979; Heinrich and others, 1981) have built their own systems. Such systems are, unfortunately, beyond the means of most laboratories.

In recent years, a class of optical instruments has been developed for optical counting of bacterial colonies. These instruments have the rudimentary pattern recognition capabilities required for those track detector applications that do not require a great deal of sophistication. In particular, they can be used for track counting and some simple size discrimination tasks. Colony counters are, therefore, not suited for use with all track detector applications. However, they can provide accurate, reproducible results for uses such as radiation dosimetry, that generally requires simple track density measurements on a large number of detectors. The principle advantages of the colony counters are that they are commercially available, and that their cost is relatively low--perhaps 1/5th to 1/10th of more sophisticated systems. They are also easy to use.

## SYSTEM DISCUSSION

At the Lawrence Livermore National Laboratory, we have incorporated one commercially available colony counter--the Biotran III made by New Brunswick Scientific Corporation--into a personnel neutron dosimetry program. Although our experience is specific to this

instrument, any colony counter should be able to provide equivalent information. No colony counters are designed to count object images using information obtained by an optical system. The optical system usually consists of a small TV camera that looks at the surface of a colony plate on an illuminated stage. The objects - colonies - are relatively large, generally ranging from 50 to several hundred  $\mu\text{m}$  in diameter. The counter is dependent on image size rather than the actual size of the objects. Therefore, they can be used equally as well to count object images presented by a TV monitor mounted on an optical microscope. In our work, the images are from roughly circular electrochemical etch tracks ranging from about 15 to 100  $\mu\text{m}$  diameter. Microscope objectives from 2X to 4X usually provide adequate magnification.

The two most important requirements for an optical counting system are that it provides reproducible, efficient track counting and that the discrimination against background artifacts be as effective as possible. The degree to which these objectives can be achieved depends on track contrast, size, shape and the inherent background features of the material in question. The electrochemical etch tracks are circular with excellent black on white contrast, and in a convenient size range requiring a modest degree of magnification. The light pinhole tracks in LR115 are also circular. The track size is smaller than ECE tracks, but still adequate for use with low magnification. The contrast characteristics are not as well-defined as those for ECE. The natural contrast is not high. Moreover, some of the tracks do not penetrate the film, so contrast continuum results. However, use of a blue-green light filter improves contrast considerably.

Simplicity in control is one feature of the colony counter. First, objects are counted only within a shaded or outlined area. In the unit we use, the size and shape [square, round or rectangular] are selectable. The track size discrimination is achieved by a size threshold control that is variable from zero to 2 mm (projected diameter on the TV monitor). These values are, in fact, nominal and should be calibrated specifically.

The contrast discrimination is achieved by a sensitivity control. As the value of sensitivity increases, the contrast necessary for counting decreases until minor fluctuations in background contrast begin to be counted. This unit has a positive-negative control that allows counting white objects on a black background (LR115). The sensitivity (contrast) plateaus for ECE tracks in CR-39 and LR115 are illustrated in Fig. 1. In practice, it is best to set the contrast discrimination as high as possible to minimize background. Then the operator should adjust the size threshold to count the smallest tracks normally seen in the detector field.

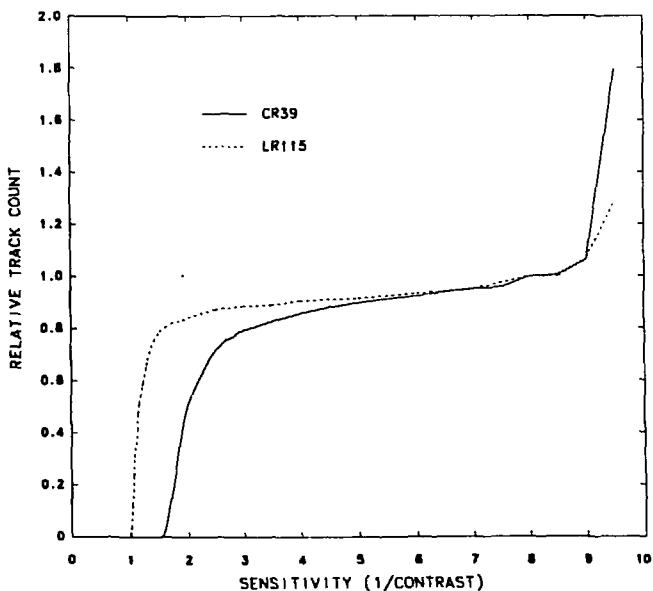


Fig. 1. Relative track count as a function of colony counter sensitivity level (contrast threshold).

An important aspect of system performance is the ability to resolve adjacent objects. As track density increases, the spatial resolution, which is analogous to radiation detector dead time, determines counting linearity. At high track densities, this results in counting losses due to track overlap. The electrochemical etch tracks do not touch. However, if the separation between their images is less than the resolving capability of the counting system, count losses will occur. This can be overcome by increasing the magnification and counting smaller fields. The limit of this approach usually occurs either when objects actually touch, or when the track images become so large that irregularities in their shape begin to cause multiple counting of single tracks. The linearity potential of this colony counter, and the value of increasing magnification is illustrated for ECE tracks in CR-39 (Fig. 2). Using a magnification of 2X, some tracks appear to go uncounted because of their small size. At 10X, the counter is capable of counting tracks accurately to densities as high as  $2 \times 10^4 \text{cm}^{-2}$ .

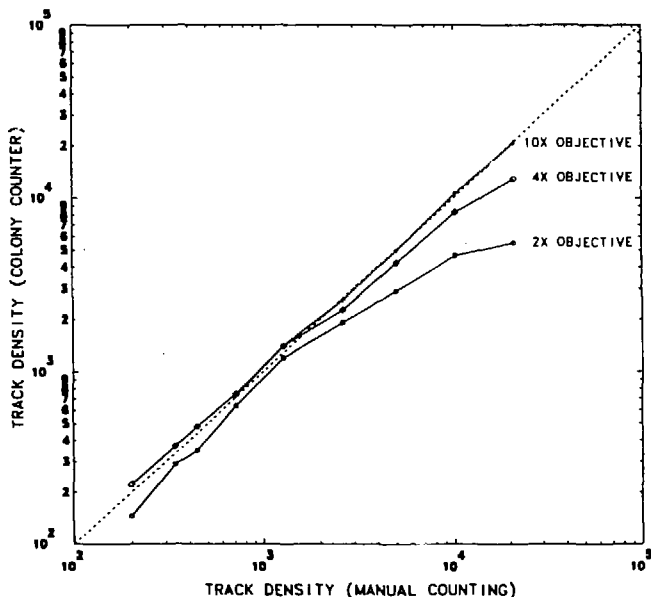


Fig. 2. Colony counter linearity for CR-39 electrochemical etch tracks.

Although not intended for object sizing, the colony counter can be used to obtain crude size information. The average object area is determined by counting a given field, then changing to a total projected area mode that provides a relative value for the total area covered by the particles that have been counted. The system magnification can be established using a calibrated reticle on the microscope slide. Thus, the user can determine the actual total track area. Dividing this by the track count gives average track area and effective average diameter. We have found, on occasion, that changes in average ECE spot size result from changes in CR-39 as received from the manufacturer. The track size can also change if certain etching parameters get out of control. Use of the average track size measurement technique provides a simple quality control measure to detect such changes.

We can determine a size distribution using the size threshold. If sequential counts are taken at increasing (or decreasing) size cut-off values, the resulting differential values provide a distribution. The quality of such distributions does not compare with data that can be obtained from more sophisticated optical analysis systems. However, there are potential applications even in ECE dosimetry. It has been proposed by Wong and Tommasino, 1982, for example, that under the proper etching conditions, the diameter of ECE tracks in polycarbonate can be related to the energy of incident alpha particles. Figure 3 presents a comparison of the size distribution obtained using the colony counter with that determined by manual counting for samples exposed to (a) 5.15 MeV  $^{239}\text{Pu}$  alphas, and (b) alphas from a mixed source of  $^{239}\text{Pu}$ ,  $^{241}\text{Am}$  (5.48 MeV) and  $^{244}\text{Cm}$  (5.80 MeV), [a 20  $\mu\text{m}$  thick polycarbonate foil was used in the original irradiation to degrade the alpha energies by approximately 2.5 MeV]. Although the peaks are not resolved as well as one might like, there is a clear difference between the two samples, and the Biotran results agree well with those obtained by manual counting.

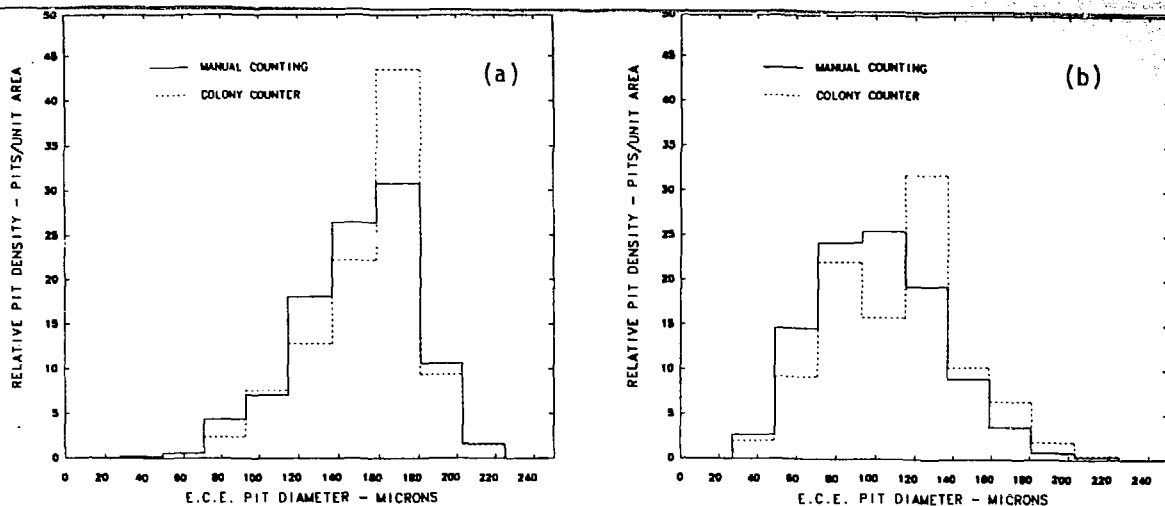


Fig. 3. Electrochemical etch pit size distributions for polycarbonate exposed to alpha particles. (Samples courtesy of J. Wong and L. Tommasino). (a)  $^{239}\text{Pu}$  alphas through  $20\ \mu\text{m}$  thick polycarbonate (b) Mixed  $^{239}\text{Pu}$ ,  $^{241}\text{Am}$  and  $^{244}\text{Cm}$  alphas through  $20\ \mu\text{m}$  thick polycarbonate.

#### SUMMARY

Bacterial colony counters have not been widely used for track counting. However, they do provide an economical alternative to sophisticated optical analyzers for applications that require reproducible track density measurements for large numbers of samples. Simple measurements of size characteristics can be made when there is little need for high resolutions. Such systems are particularly well suited for neutron and alpha dosimetry work, particularly if electrochemical etching or some other track enhancement method has been used.

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