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SUFFIELD MEMORANDUM

NO. 1233

LASER DETECTION AND MAPPING OF BIOLOGICAL SIMULANTS II.

PRELIMINARY CONCENTRATION AND PARTICLE SHAPE RESULTS (U)

by

J. Ho, M. Spence, B.T.N. Evans* and G. Roy*

Project No. 351SQ

February 1989

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* DREV Defence Scientists

ABSTRACT

The Laser Cloud Mapper (LCM) was designed at DREV to measure and map smoke particle concentrations. This instrument was optimized for larger particle sizes of grenade smoke (>15 μm). Since most CB aerosol particles are less than 15 μm in size, it was of practical interest to determine if the LCM could detect CB aerosols. A BW simulant aerosol (bacterial spores) was generated in the field and the LCM was used to measure light scattered by the particles. Various aerosol concentrations were produced to determine low detection limits of the instrument. Preliminary observations indicate that this device was able to detect the simulant aerosol. Lower limits of detection were about 200 to 400 viable spores/liter. Furthermore, with the aid of polarizing filter attachments, the LCM was able to differentiate between the shapes of solvent derived particles (spherical) and spores (elliptical). These capabilities may have important implications for future designs of remote CB aerosol detectors for research and military applications.

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INTRODUCTION

1. DREV had constructed a laser-based light scattering device capable of remote detection of aerosol particles (1). The Laser Cloud Mapper (LCM) was designed and refined at DREV to be a portable field unit. At present, the instrument can generate raw data as a stand alone device. Data reduction, using advanced algorithms (2), takes two days and the final presentations provided comprehensible contour maps of different concentration ranges. If the extinction coefficient of the cloud material is known, then absolute concentration units can be plotted. This capability has important implications for remote detection of CB aerosols.

2. Recently the DREV LCM was modified with a laser polarizing system (3). It is beyond the scope of this report to discuss the mathematical derivations, but this added feature was postulated (3) to confer shape discrimination capabilities to the system. Biological

particles have well defined shapes and could be used to verify this capability. For example, bacterial spores have elongated shapes with dimensions of 0.7 by 1.5 µm (5).

3. All of the above were compelling reasons for DRES and DREV to undertake the task of determining if the LCM or a modified instrument could function as a remote CB aerosol detector. Initially the following points were raised:

a. since the LCM was not specifically optimized for CB aerosol detection (0.5 to 15 µm), could it detect a simulant BW aerosol;
b. if detection was possible, what would the lower concentration limits be;
c. could the system distinguish elongated particles, a characteristic of most BW agents, from spherical particles; and
d. were there unknown factors which might render the device unsuited for the task.

4. It was also expected that additional information would be obtained to assist the original LCM maker in designing an optimized version of a remote CBW aerosol detector.

MATERIALS AND METHODS

5. The overall plan was to present various types of aerosols at different concentrations to assess the detection capabilities of the LCM. To generate a representative size aerosol cloud, a Micronair disseminator was used. This was chosen for its simplicity of operation as well as the broad particle size spectrum of its output. A steady wind was required to carry this cloud across the LCM line of sight.

Light scattered by the aerosol particles was collected by the LCM optics and detections system and the signals were stored for later analysis. Complex algorithms were used to convert raw signals to derive useful data, presented as contour plots. The ability to produce user comprehensible data plots is the most critical attribute of the LCM hardware and software.

BW Simulant

6. A spore suspension of Bacillus subtilis Var. niger species globigii (BG) was used as the simulant. Viability of the sample was better than 1 x 10^9 cells per ml (100% BG slurry). Lower concentrations used in the experiments were diluted with tap water. Tap water, alone, was used to produce a control aerosol.

Aerosol Generator

7. A Micronair generator (model AU7000, Micronair Limited, Bembridge Fort, Sandown, Isle of Wight, PO36 8QS, England) provided a continuous, polydisperse aerosol. This unit was factory equipped (special order) with 110 VAC motor which drove a 18 cm propeller at maximum speed. The sample suspension was delivered at 0.6 L/min from a pressurized plastic bottle. This container was mildly pressurized by a small adjustable air pump (model MT3300, Campbell Hausfeld, Harrison, Ohio, 45030). By adjusting the air pressure control valve, a steady flow of sample was delivered to a dispersion wire cage situated in front of the spinning propeller. Dispersion of the aerosol was achieved by the propeller as well as by the prevailing wind (westerly at 10- 25 KPH). The dispersion source was located 2 meters above ground. Electrical power to drive all the equipment was supplied by a 2500 watt Honda generator (model E2500C, Honda Canada Inc., Scarborough,

Ontario, M1B 2K8).

Biological Aerosol Sampling

8. In determination of the hazards from BW aerosols, the viable cell content is an essential piece of information. This information is also useful in interpreting light scattering results by particles in general. In order to relate LCM results to BW content, it was necessary to estimate the presence of viable spores. A dichotomous sampler (DS), also called a virtual impactor, was used to collect particulate aerosols (Series 245, Sierra Instruments, Inc. Carmel Valley, CA. 93924). This instrument was selected since it has been found to be highly efficient for inhalable particles (4). Subsequent testing of the DS with BG aerosols at DRES confirmed that its collection efficiency was equal to or better than standard glass impingers (11).

9. The aerosol stream was drawn through a size exclusion intake manifold with an upper size limit of 15 μm. Particulate samples were collected on filters held in a 20 slot carousel. Each slot contained two filters which corresponded to two size groups sorted by the virtual impactor. The groups consisted of large particles from 2.5 to 15 μm and small particles of 2.5 to 0.5 μm. Particles greater than 15 μm were excluded by the intake manifold. Efficiency of collecting particles less than 0.5 μm was a function of the type of filters employed. A cost effective borosilicate microfiber filter was selected for this purpose (Grade GA55, Cat. no GA5537MM, 37 mm diameter, Micro Filtration Systems, Dublin, CA. 94568). This filter was chosen for its ease in resuspending collected particles in distilled water as well as for its collection efficiency for small particles.

10. Modifications to the electronic controls of the DS were made to speed up sampling times (selectable timing resolution in seconds rather than hours). External timing signals were provided by a programmable timer (Chrontrol, Linburg Enterprises, Inc., San Diego, CA 92126). This timer was set to output a 110 volt pulse (1 sec) at 2 min intervals to a relay switch installed in the DS sample compartment. This provided a momentary contact closure of the sample position advance control switch. This action caused the sample tray to move forward one position. Actual sampling time was 85 seconds. The rest of the time (35 sec) was taken up by the slow mechanical movement of the sample change mechanisms.

11. The 2 min interval timing was selected to allow collection of samples at optimal time resolution. Given the maximum of 20 sample slots, half of these were allocated to controls (five before the aerosol spray and five after) and the rest to samples. The duration of sample spray was dictated by economics of BG slurry expenditure. Preliminary testing indicated that the DS was able to collect sufficient viable spores within this timing interval.

12. Another modification to the standard DS operating procedure was made. In order to avoid interfering with the LCM laser beam, the instrument was used without its factory supplied elevation stand. In this configuration the aerosol intake was only one meter above ground.

Assay Of Viable Cells

13. Particulate aerosol samples collected on filters were stored dry in capped glass tubes. These were transported back to DRES for microbiological assays. Distilled water (20 mL) was added to each sample tube containing a filter. The capped tubes were then shaken for

10 minutes by a wrist action shaker (model 75, Burrel Corp., Pittsburg, PA) to resuspend the BG spores. The glass fiber slurry was strained through a wire gauze disk to recover clarified filtrate containing BG spores. Viable spores were enumerated by the spiral plating technique (10). Liquid samples were applied on to standard nutrient agar plates with a spiral plater (model CU, Spiral Systems Instruments Inc., Bethesda, MD). As the petri plate rotated, this device laid down a sample stream of varying volumes along a spiral path. Starting from the center where the highest sample volume was applied, the stylus progressed towards the perimeter ejecting ever decreasing volumes until 45 µL was exhausted. The plates were incubated over night at 30°C. A laser-based spiral colony counter with an integrated data processor (model 500A and model 800 respectively, Spiral Systems Instruments Inc.) were used to calculate the viable spores in the original sample.

Aerosol Concentration Calculations

14. The number of viable spores per liter of aerosol was determined from equations [1] and [2] using the following information: fine particle flow rate (FF)= 17 L/min, coarse particle flow rate (CF)= 1.7 L/min, number of spores on fine filter (FN) and number of spores on coarse filter (CN) both determined from microbiological assays, and sampling time (T)= 1.417 min.

$$\text{Fine aerosol concentration} = FN/T \times 1/FF \text{ number/L} \quad ..[1]$$

$$\text{Coarse aerosol concentration} = CN/T \times 1/CF \text{ number/L} \quad ..[2]$$

Aerodynamic particle sizer (APS) measurements

15. It was not possible to perform aerodynamic particle sizing measurements of the test aerosol at DREV. However, using identical

experimental conditions at DRES, the BG aerosol was characterized by an APS (model 3300, TSI, St.Paul, MN). This instrument was located in a general purpose shelter sitting on a 1.5 ton M104 trailer (DND publication no. C-30-570-000/JD-000, pages 7 and 13). This shelter system housed the APS in a temperature controlled environment. An aerosol intake manifold, similar to that described for the DS, located 5 m above ground level was attached to the APS. A sampling protocol similar to that described later for the DS was used here.

Scanning Electron Microscopy (SEM)

16. These experiments were done at DRES. An Andersen sampler was used to trap aerosol particles generated from a Micronair generator with 100% BG slurry (6). Aluminum tape (model EE369-1, Marivac Ltd) was placed on each Andersen collection stage to trap the appropriate size particles for scanning electron microscopic analysis. The tape was oriented with its glued surface directed at the aerosol stream for efficient trapping of particles. Standard SEM techniques were used to process the material for examination (8). Samples were shadowed with gold-palladium (model Hummer II, Technics Inc.) to enhance image contrast. A Hitachi SEM (model S-450LB, Hitachi Inc.) was used to examine the shadowed samples.

The LCM

17. Technical personnel from DREV operated the LCM and its associated systems. The technical details of the apparatus have been published (1) along with the polarizer modifications (3). The laser and its associated control electronics were housed in a large trailer. A beam of 1.06 μm wavelength laser light was projected from a window on the side of the enclosure. The scanning pattern of this beam was

determined by preprogrammed parameters which controlled a moving mirror. Light scattered by particles was measured by a sensitive solid- state detector. Analogue signals from this detector was digitized and stored on disks. Data reduction and plotting were performed in the laboratory, and could take several days as complex analytical procedures were required to extract the maximum information from the raw data. Further technical details were described in reference (3).

Experimental Layout

18. Details of the test site at DREV were described in reference (3). The LCM scanned an of 90 degrees with a beam elevation of 10 degrees from horizontal (Figure 1). The Micronair aerosol generator was located 100 meters upwind of the LCM. For collection of BW particles, the DS was located 75 m directly downwind from the aerosol source. As mention earlier, the aerosol intake of this sampler was about 1 m above ground level.

19. In a typical experiment, the DS collected 5 background samples (control). The Micronair was energized 10 min after zero time to provide the aerosol source. The DS collected 15 aerosol samples at 2 min intervals. At 11 min after zero time the first LCM scan was initiated and this was repeated every 2 min for 8 to 10 consecutive samples.

RESULTS AND DISCUSSIONS

20. Several biological aerosol sampling considerations needed to be addressed if the collected data were to be meaningful. First, conventional biological samplers which run continuously yielding only a

single time-integrated (10-20 min) sample would not be appropriate in this application. Data so obtained could not reflect dynamic aspects of the aerosol cloud. Since the LCM time resolution was in seconds, a better time-resolved BW sampler was required. Second, the BW sampler should also be able to acquire multiple samples unattended. This was essential as during passage of an aerosol cloud, human intervention of sampling equipment was not practical. The instrument that met these requirements was the dichotomous sampler.

21. Using this sampler, multiple samples of viable spores were collected from a passing aerosol cloud. Each sample represented a time integration of 85 sec. Previous DS experiments with Micronair-generated BG aerosol provided data to optimize on this time (11). It should be acknowledged that this time interval was still much longer than that of a LCM scan (<2 sec per horizontal slice). Because of this discrepancy, the measured biological aerosol concentration profile was not expected to be identical to that detected by the LCM.

22. This turned out to be the case. A typical map of a biological aerosol cloud could be represented as a two dimensional plot of viable spores per liter versus time (Figure 2). Each point depicted the time averaged (85 sec) aerosol concentration during passage of the cloud at the point of sampling. It was essential to demonstrate that the control experiment picked up no environmentally-derived organisms that might distort recovery and identification of the simulant. As shown in Figure 2, there was no background BG aerosol in the controls (first 10 min of the experiment). After 10 min, the Micronair generator began to disseminate an aerosol from a 100% BG slurry. Roughly 4 min later the DS detected the presence of the passing cloud. This was followed by a rapid increase in aerosol concentration. An apparent

peak in concentration was observed at about 14 min from the start of the Micronair. After the aerosol source was turned off, no more BG simulant was detected. The decay time was quite rapid, within the 2 min sampling time.

23. Given that this experiment had only one biological detector, the results shown in Figure 2 must be qualified. It was obvious that this detector sampled only a small portion of the whole cloud. Although the DS was sampling at 1 m above ground level, not an ideal location for representative measurements, the presence of a viable spore aerosol was demonstrated.

24. With these caveats in mind, the following interpretations could be made from Figure 2. The aerosol cloud did contain viable BG spores, but all the viable spores were found in the coarse sample filters i.e. >2.5 μm particles. The maximum observed aerosol concentration of about 8000 spores/L was probably a low estimate. Considering the nature of the experiment, rounding this value to 10000 spores/L would be a better estimate. This level was similar to that of simulated BW aerosols used in the laboratory (9), which could represent the maximum aerosol concentration detected by the LCM.

25. Few, if any, spores were recovered from the fine sample filters. It is likely that the smaller lighter particles were carried higher up in the air, and few small particles were collected at 1 m. Figure 3 shows that the proportion of particles emanating from the Micronair was skewed toward the larger diameters. The results indicated that the Micronair did indeed produced a broad spectrum of particles covering the 1 to 10 μm diameter size range.

26. The corresponding LCM profile of this aerosol cloud (from

100% BG slurry) is shown in Figure 4. This figure (from (3)) represents a horizontal concentration slice taken from close to the middle of the cloud (5 m above ground) and was obtained about 10 min after the start of the Micronair generator. Certain distinctive characteristics could be observed. Higher cloud concentrations were associated with the area close to the source. Conversely, lower concentrations were seen farther away from the source. It can be seen that in the area where the cloud was being studied, there was considerable discontinuity in particulate concentrations as opposed to a gradual gradient. The observed rapid drop in recovered viable spores at time = 26 min (Figure 2) could be due to a discontinuity in the cloud.

27. Figure 4 also shows that the composite cloud consisted of individual puffs. Each puff of aerosol exhibited a concentration gradient, the higher concentrations being close to the center. This figure shows the ability of the LCM to obtain dynamic details of a cloud. With this capability, the LCM is invaluable in studies of the interaction dynamics of aerosols with the environment. Such information could be useful in modeling and prediction experiments. In contrast, the DS results revealed that it was more suited to measuring average concentrations at different time intervals. Thus the results from the two detectors are complementary.

28. An attempt was made to determine the low concentration detectability limit of the instruments. Figure 5 shows an aerosol profile as resolved by the DS when presented with a cloud derived from a 33% BG slurry (by volume). An average high concentration of about 1600 viable spore/L aerosol was measured. The average low concentration was between 200 to 400/L. The LCM could resolve this cloud with no difficulties (3).

29. Further experiments with lower slurry concentrations revealed that the DS was not configured optimally to detect aerosols generated from 10% slurry preparations. This limitation prevented direct biological verification of the lowest detectability level for the LCM. Thus, at the present state of experimentation, the interim lower detectable concentration would be 200 to 400 spores/L. It must be emphasized that this limitation is not due to instrumentation deficiencies; rather it is because the DS was not optimized to detect lower BG aerosol concentrations. Further refinements in DS performance (for example, increasing sampling time) could improve its detection capability for this size range by an order magnitude. It would then be possible to confirm the LCM performance.

30. A comparison of the DS results from two experiments using two slurry concentrations revealed that the lower the slurry concentration the lower the aerosol concentrations (Figure 6). In Figure 6, the aerosol concentration was plotted as a logarithm (base 10) to accommodate the dynamic range. This observation confirmed the LCM results (3) which also indicated similar concentration discriminations. Furthermore, it was shown that the LCM could easily detect an aerosol generated from a 10% slurry.

31. Data from varying slurry concentration studies were summarized in Figure 7 where scattered light signals from different experiments were analyzed in conjunction with polarized light (3). In this diagram, an increase in the Y axis value represented greater deviation from particle sphericity. This was illustrated by a number of spherical aerosol types presented on the X axis. Oil, as well as tap water (no BG control) which served as the experimental control, were assumed to produced mostly spherical particles (3). These samples registered low values on the Y axis scale.

32. In contrast, strong signals were registered from the aerosols derived from 10% and 100% BG slurries. This increase in depolarized signal could be a contribution from individual elongate spores which measure about 0.7 by 1.5 μm (5). However, between these two sources, stronger signals were registered from aerosols of 10% slurry (Figure 7). This was rather a puzzling observation as it was expected that a greater aerosol concentration would yield a correspondingly greater depolarized signal.

33. The explanation might lie in the way spores interact during aerosolization. When a BG slurry is sprayed from the Micronair generator, a variety of particle sizes can result. The particle size range of the aerosol, illustrated in Figure 3, was 1 to 10 μm in diameter. By trapping these particles with a multistage Andersen sampler (6), the isolated large particles were seen to consist of large numbers of individual spores (Figure 8). In this example, several hundred spores made up a typical 9.5 μm particle clump. Also, during sampling, the particle broke up on impact, scattering its contents as a "splash" of about 15 μm in diameter. Similarly, a smaller (3 μm diameter) clump, collected from a lower stage, was shown to consist of fewer spores (Figure 9). In this Andersen sampling experiment, no particles were found on stages lower than 3 (<3 μm particles). This was mainly due to difficulties in trapping, locating and identifying really small particles by SEM.

34. The individual spores were compared to other size markers in Figure 10. Note that some spores also appeared as pairs while others formed clumps of 4 to 6, despite the fact that this sample was collected from an aerosol generated with a Collison aerosol generator (7) which preferentially produces individualized particles. This and other evidence presented above support the idea that in an aerosol, a

proportion of BG spores have a tendency to form aggregates of various sizes. The nature of aggregation could be a function of generator type and/or slurry concentration. Other possible factors, not easily verifiable, could be extracellular polysaccharide material, produced by vegetative cells and not completely eliminated during spore harvesting, inducing spores to stick to each other.

35. This tendency of high concentration spore suspensions toward aggregate formation may explain the observations in Figure 7. It is logical to speculate that such aggregates, being somewhat spherical, may not contribute much to depolarization. Moreover, due to their relative size, they may serve to block some of the depolarized light scattered from single particles. Conversely, a dilute spore suspension may generate greater numbers of single particles which depolarize the incident laser beam. As there are fewer aggregates in the aerosol, the scattered light suffers little to no blockage on its way back to the detector. All these possibilities remain to be tested in the laboratory and the field.

CONCLUSIONS

36. The LCM was found to be capable of detecting a BW simulant aerosol. Actual viable spore measurements verified that the lower detection limit was for aerosol concentration of 200-400 spore/L. At this level, the instrument could be useful as a remote CBW aerosol detector. Future work should be directed at verifying if the instrument is capable of performing at lower concentration limits. Convincing evidence was presented to suggest that the LCM could measure degrees of particle sphericity. There were distinct depolarized signal differences derived from BG aerosols versus known spherical aerosols (controls). However, more work would be required to relate these

observations to BG aerosols of varying concentrations.

37. A new area of aerosol research was identified from these studies. The relationship between BG slurry concentration and the tendency for the particles to form aggregates during aerosolization needs further investigation. Also to be elucidated would be the aerosol size distribution characteristics as affected by different aerosol generators.

38. The data reduction and presentation capability of a future detector system would have to be better integrated. By rewriting the software to run on a fast microcomputer (supermicrocomputer), it might be possible to generate real time displays of aerosol clouds in absolute concentrations. This capability would greatly enhance research in CB aerosol detection.

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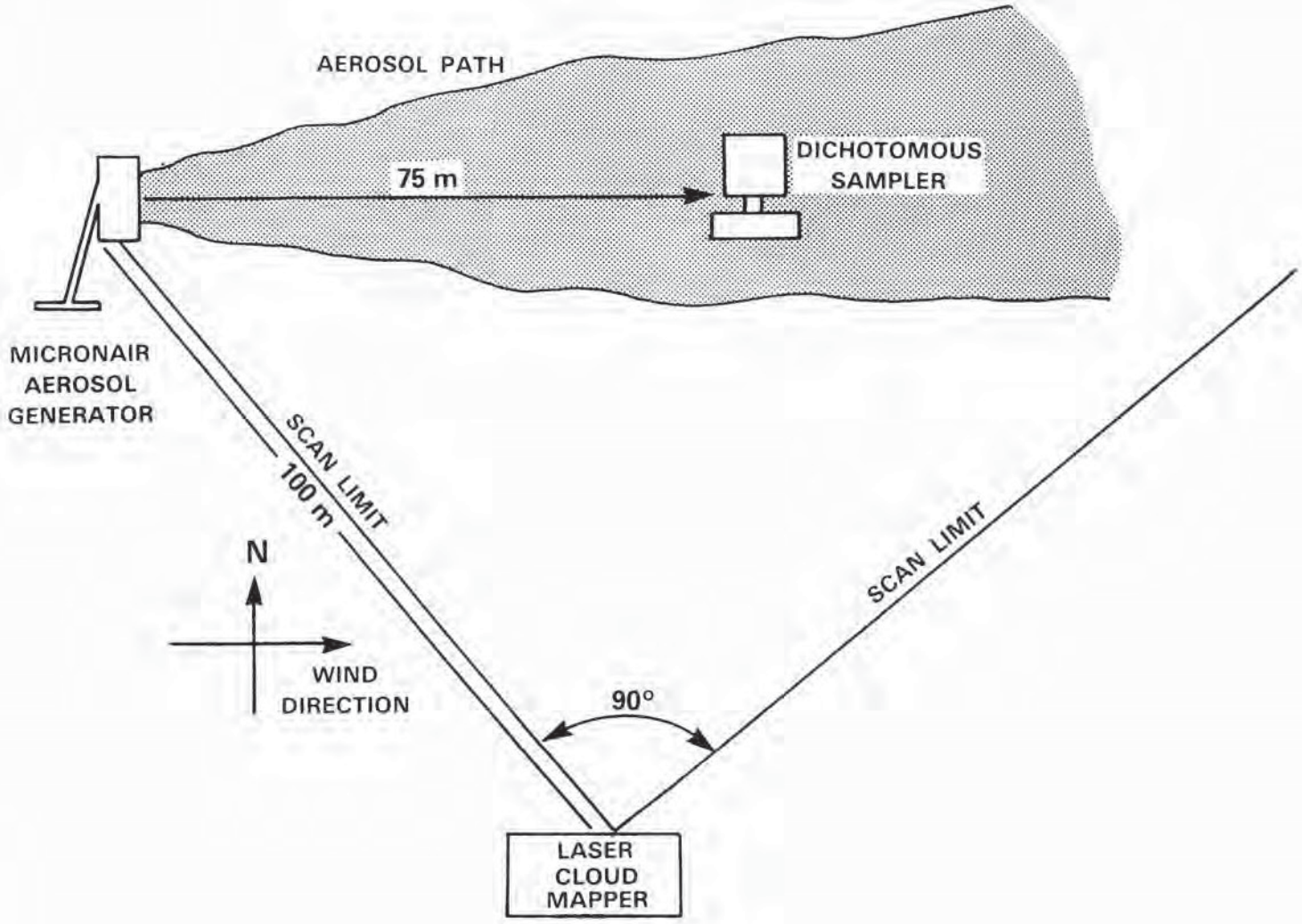

Figure 1

EXPERIMENTAL LAYOUT

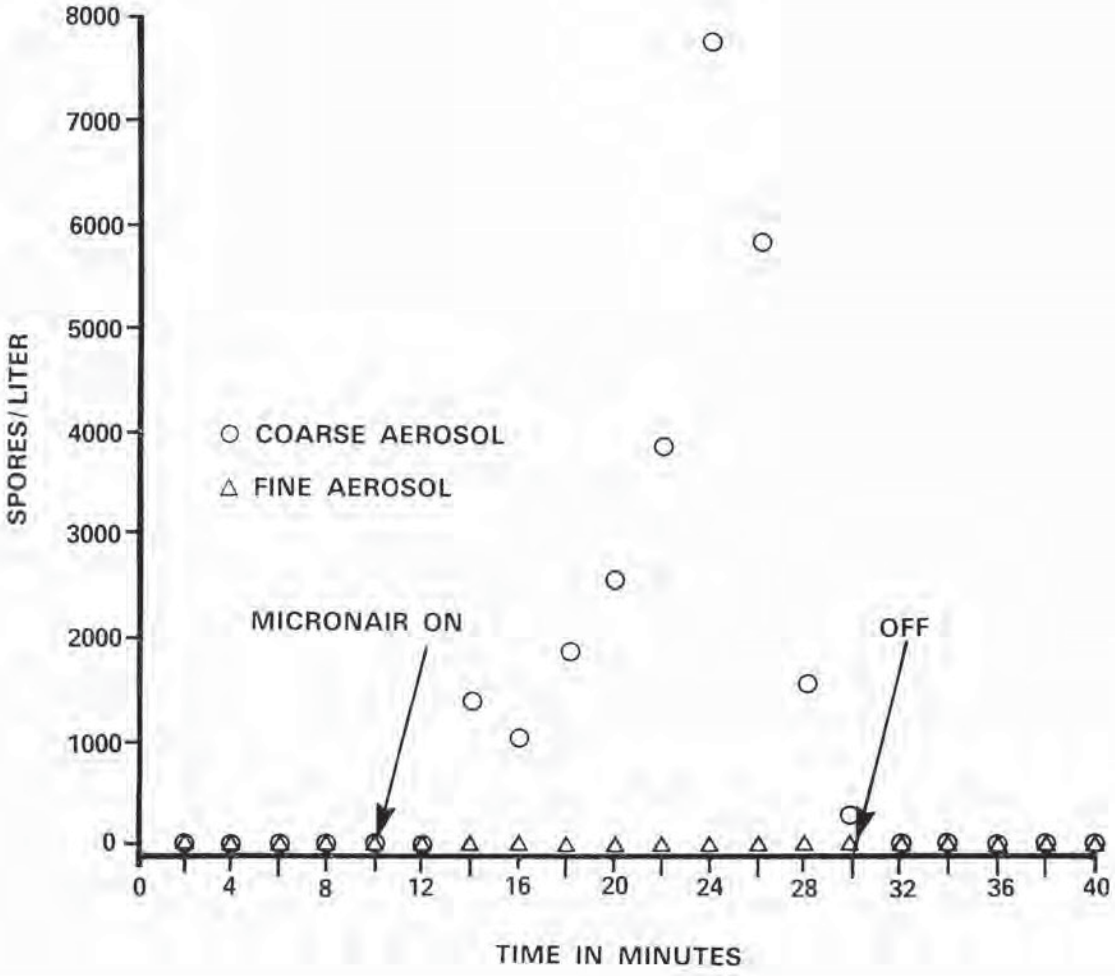

Figure 2

DOWNWIND AEROSOL CONCENTRATION FROM 100% SOURCE STRENGTH

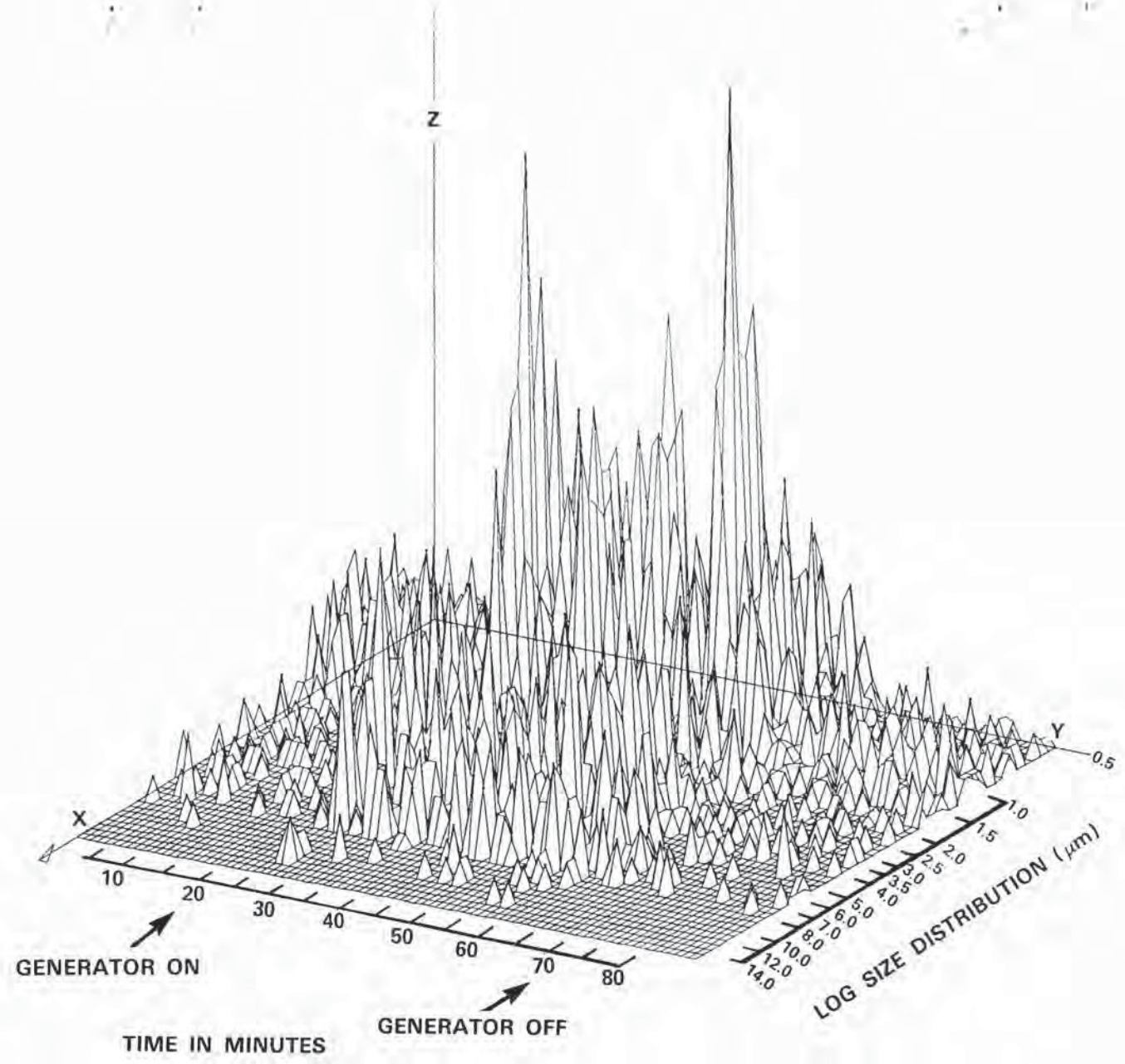

Figure 3

PARTICLE SIZE DISTRIBUTION OF BG AEROSOL
GENERATED WITH A MICRONAIR

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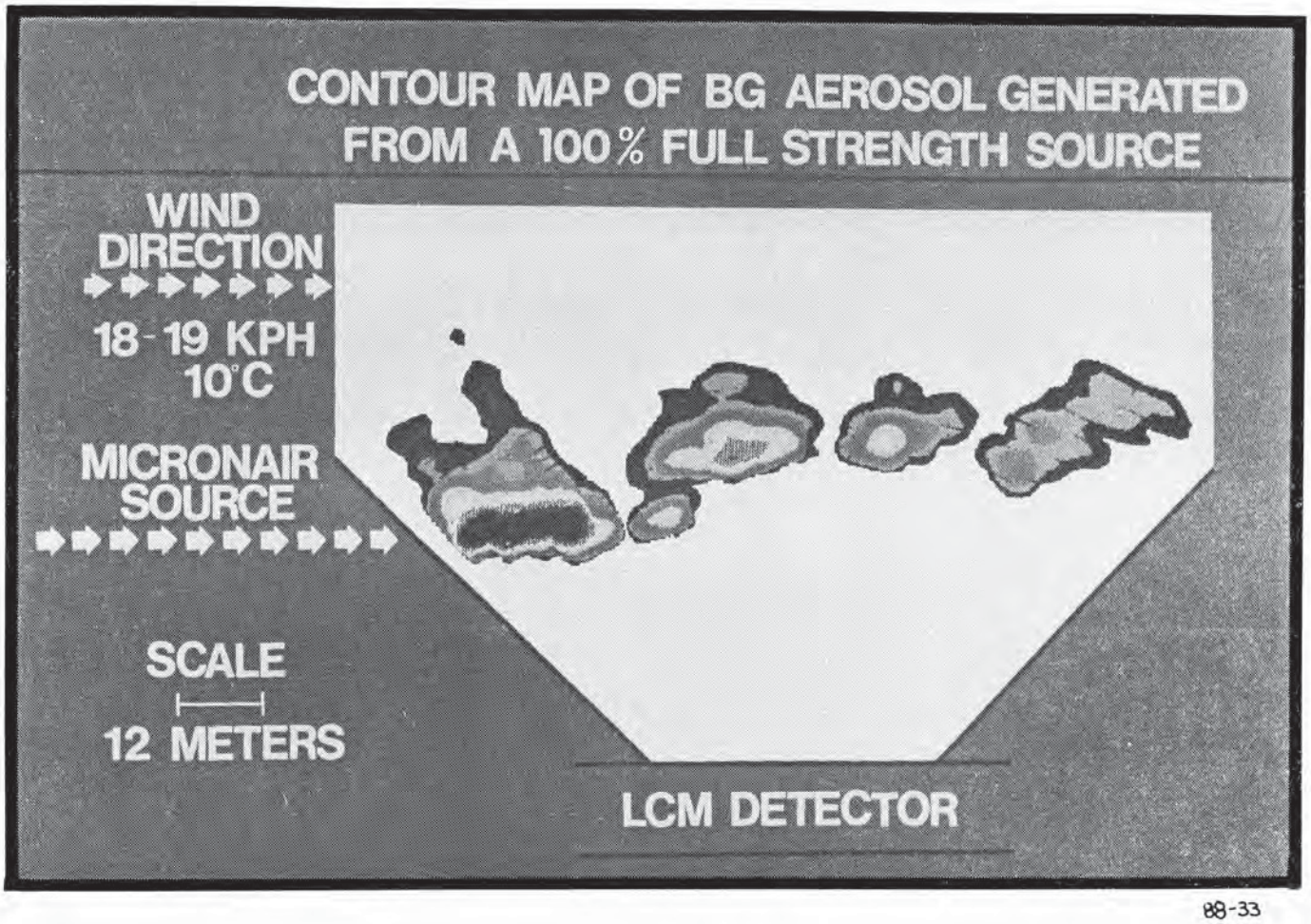

Figure 4

CONTOUR MAP OF BG AEROSOL GENERATED
FROM A 100% FULL STRENGTH SOURCE

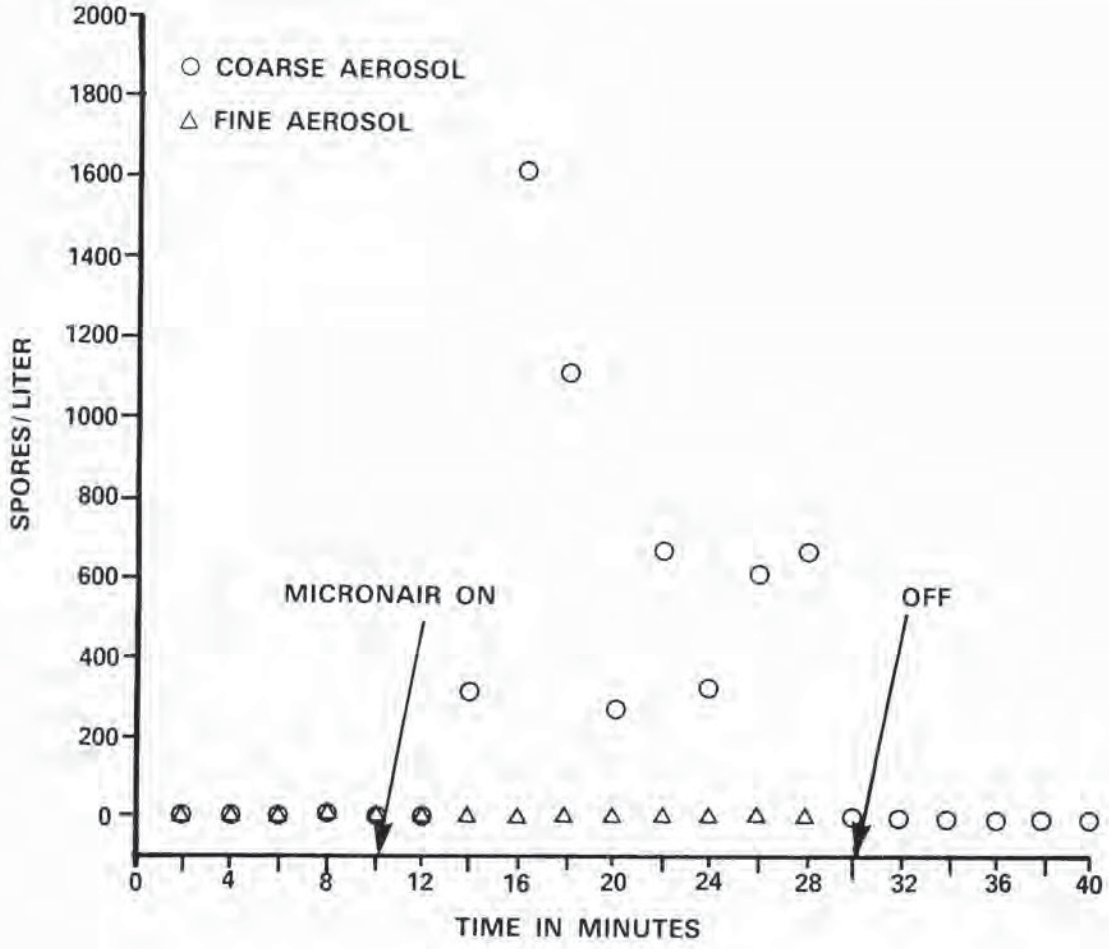

Figure 5

DOWNWIND AEROSOL CONCENTRATION FROM 33% SOURCE STRENGTH

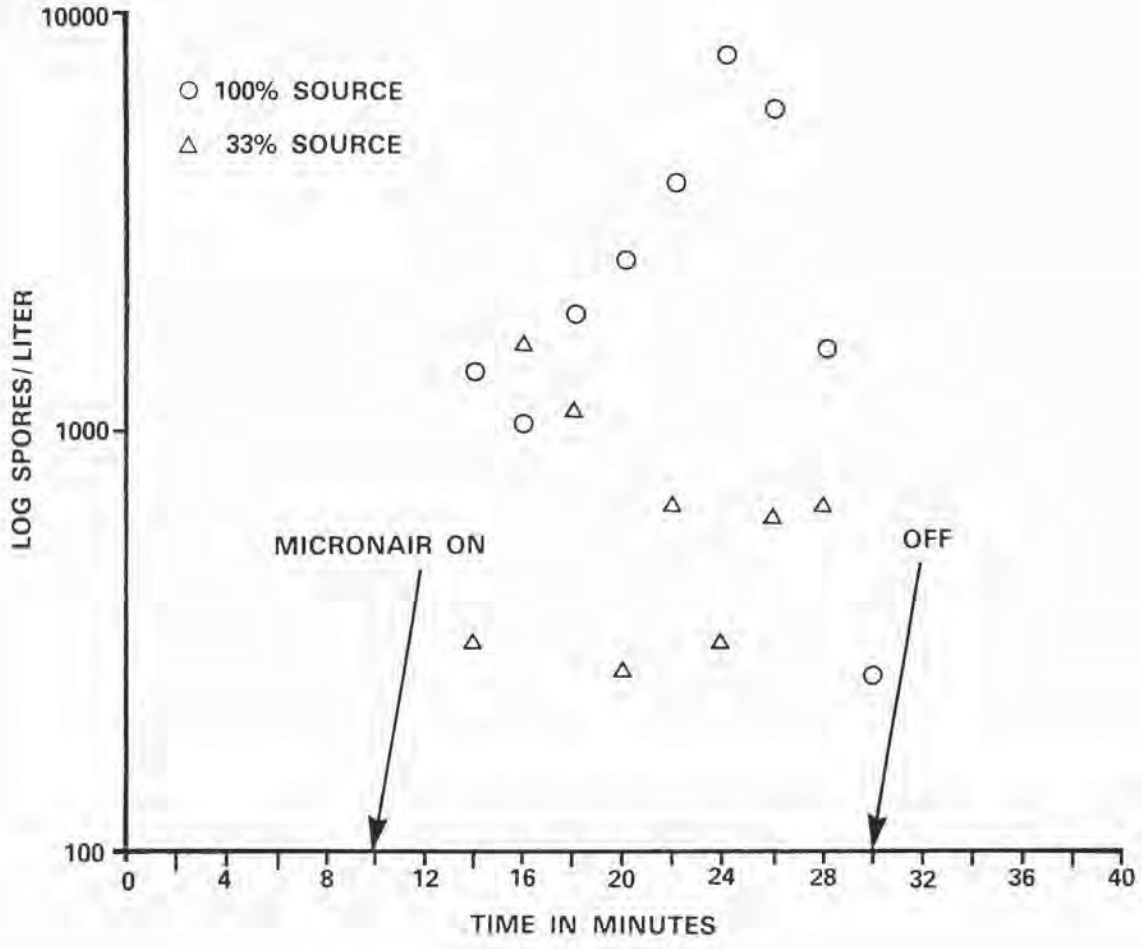

Figure 6

DOWNWIND AEROSOL CONCENTRATION AT TWO SOURCE STRENGTHS

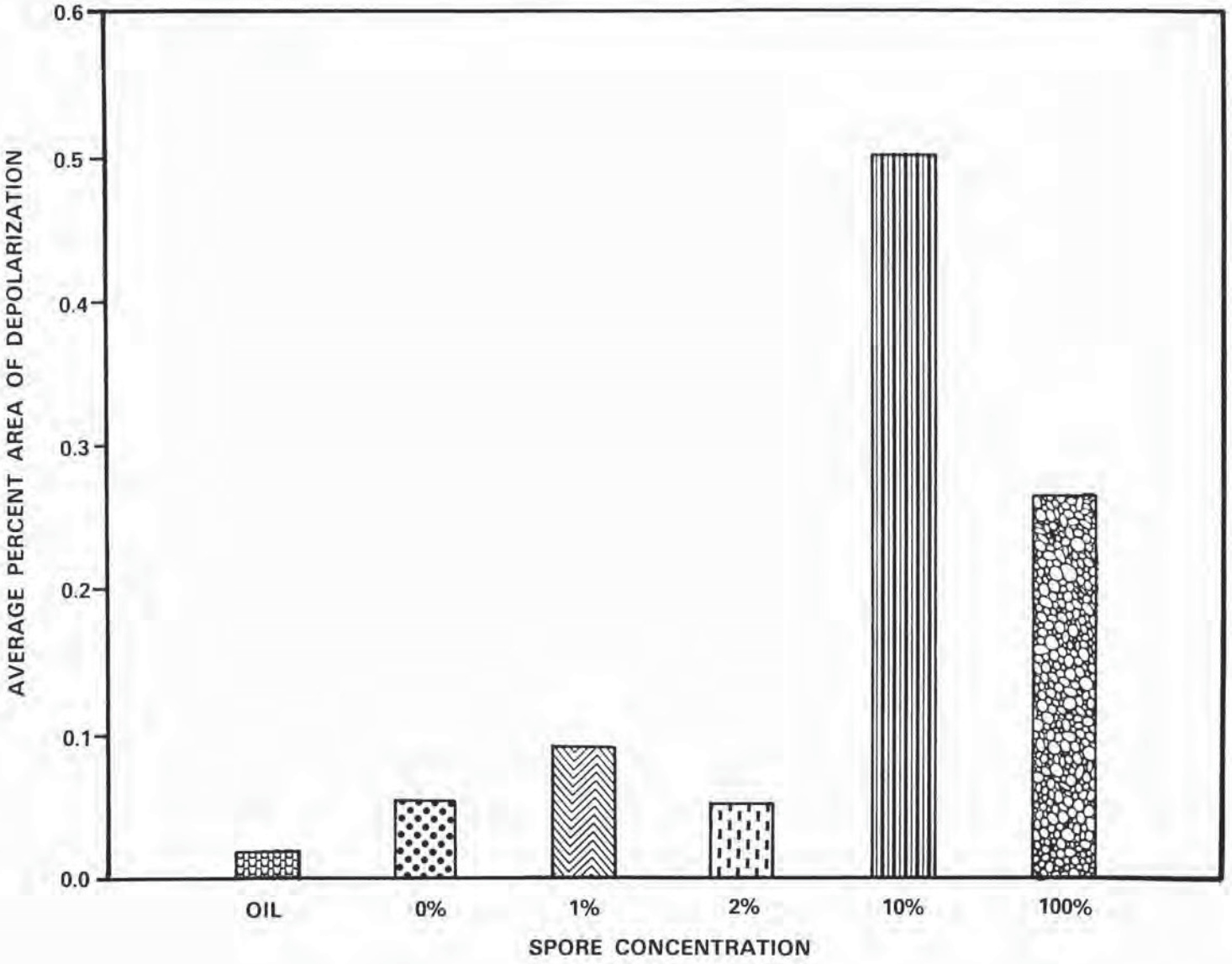

Figure 7

COMPARISON OF DEPOLARIZED SCATTERED LIGHT SIGNALS FROM DIFFERENT TYPES OF AEROSOL PARTICLES

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Figure 8

SCANNING ELECTRON MICROGRAPH OF AN AEROSOL PARTICLE,
DIAMETER 9.5 μm,
COLLECTED WITH AN ANDERSEN SAMPLER

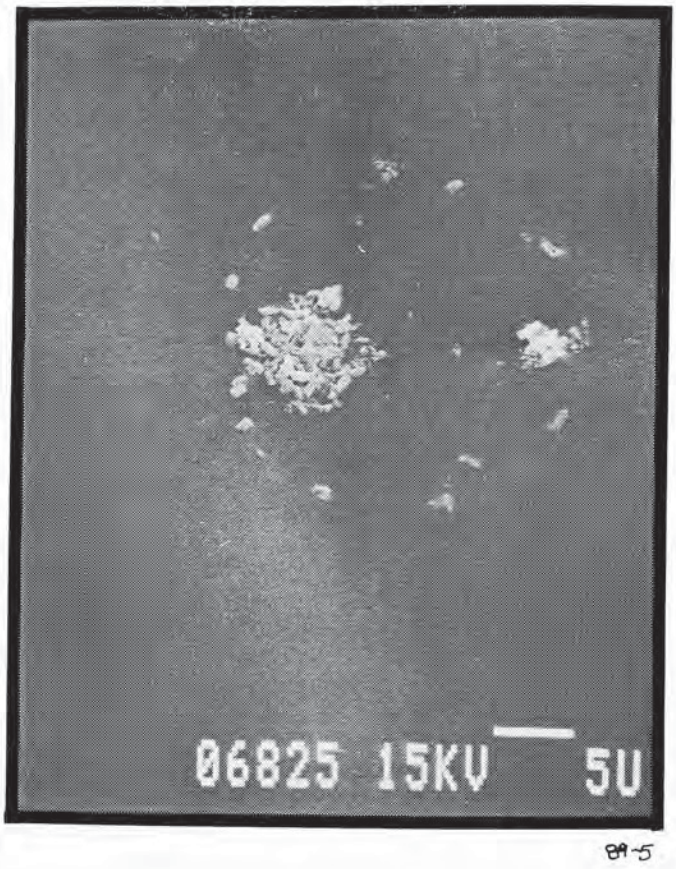

Figure 9

SCANNING ELECTRON MICROGRAPH OF AN AEROSOL PARTICLE,
DIAMETER 3.0 μm,
COLLECTED WITH AN ANDERSEN SAMPLER

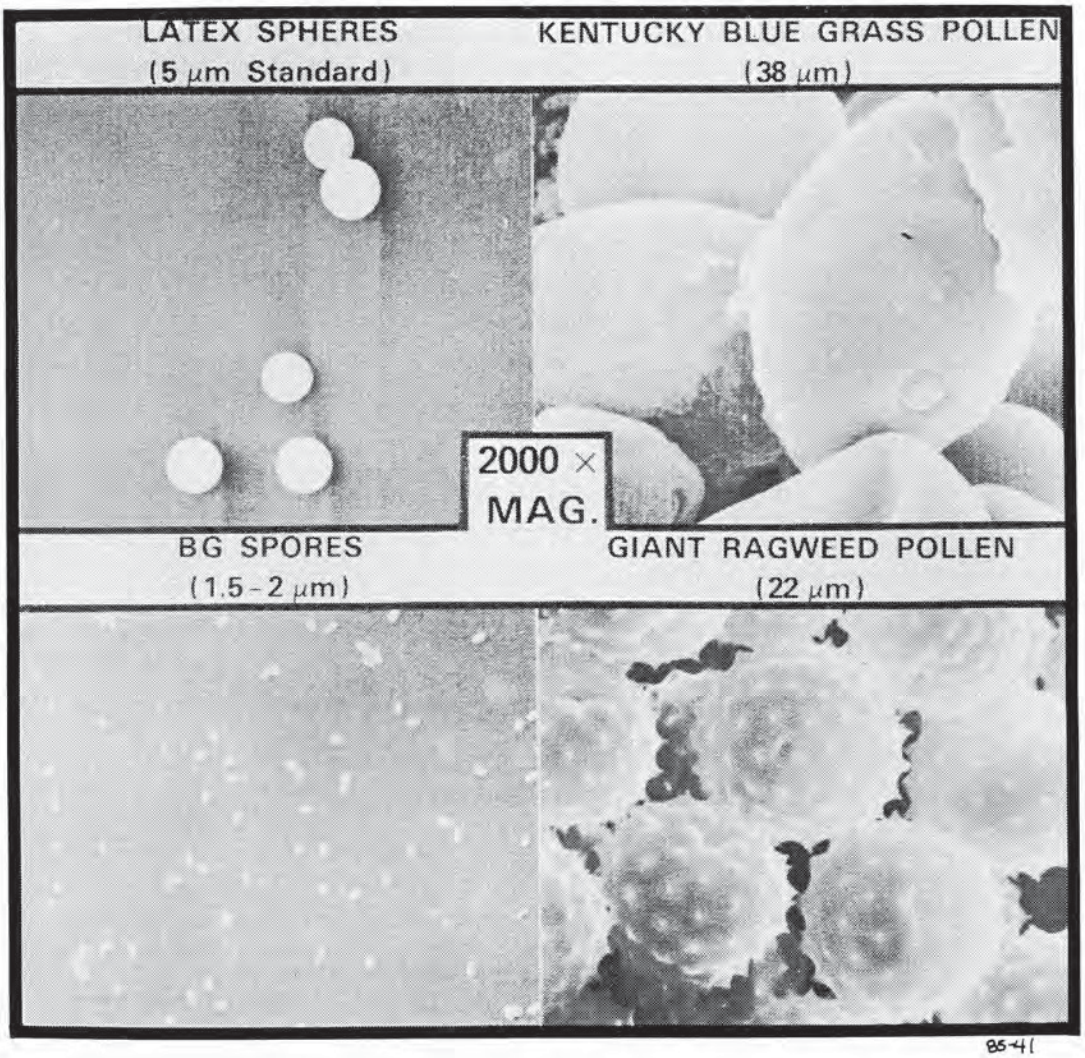

Figure 10

COMPARISON OF PARTICLES OF DIFFERENT SIZES

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The Laser Cloud Mapper (LCM) was designed at DREV to measure and map smoke particle concentrations. This instrument was optimized for larger particles of grenade smoke (>15 μm). Since most CB aerosol particles are less than 15 μm in size, it was of practical interest to determine if the LCM could detect CB aerosols. A BW simulant aerosol (bacterial spores) was generated in the field and the LCM was used to measure light scattered by the particles. Various aerosol concentrations were produced to determine low detection limits of the instrument. Preliminary observations indicate that this device was able to detect the simulant aerosol. Lower limits of detection were about 200 to 400 viable spores/liter. Furthermore, with the aid of polarizing filter attachments, the LCM was able to differentiate between the shapes of solvent derived particles (spherical) and spores (elliptical). These capabilities may have important implications for future designs of remote CB aerosol detectors for research and military applications.

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Keywords: Laser detection; remote aerosol detection; particle shape; BW aerosol; cloud concentration; BG spores.