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General Mills

FINAL REPORT
(FEASIBILITY STUDY)

MICROSCOPIC SYSTEM FOR MARS STUDY PROGRAM

CONTRACT NO. JPL 950123

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ABSTRACT

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The results of a five-month study program have indicated that a feasible design concept can be developed for a remote, automatic microscopic system of Martian life-detection. Environmental samples would be collected, refined and microscopically observed; video-micrographs of specimens with a high probability of containing biological constituents (if indeed, they are present) would be transmitted back to Earth. The individual study phases have included environmental factors, inferred characteristics of Martian microorganisms, sample collection, sample processing, microscopic observation and detection, and overall system analysis.

We have concluded that representative samples can be best obtained by means of an inertial impactor which draws in aerosols from the atmosphere and aerosolizable particles from the Martian surface. Density fractionation in a suitable flotation liquid would then be carried out in a miniature centrifuge chamber to separate the lower density (and presumably biological) particles which would be removed from the chamber by a simple fluid mechanical system actuated by changes in the centrifuge speed. The refined particles would be fractionated by filtration into two size ranges of 0.5 to 10 microns and 10 to 50 microns in diameter. The larger filter-collected particles would be transported by a simple tape mechanism to a low-power microscope. The smaller particles would be collected on a highly concentrated area of an optical viewing surface by means of centrifugal impaction, micro-area filtration or electrohydrodynamic precipitation. The small particle collectors could be suitably integrated into the microscope design to eliminate the need for transport to the viewing stage.

The microscope subsystem comprises separate modules for specimen observation: a simple 100X visible light microscope unit with fixed focus and fixed field to observe the filter-collected larger specimens, and a more

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complex 1000X ultraviolet immersion microscope with limited autofocus (and possibly horizontal scanning) capabilities to observe the smaller particle specimens. A microspectrophotometric detector can be incorporated into the high-power microscope for biological analysis of differential ultraviolet absorption characteristics. A critical concept which greatly simplifies the optical design is the concentration of refined samples into a viewing area which is approximately equivalent to the microscopic field-of-view.

The outline of a recommended experimental study program is also presented. Further laboratory and theoretical studies would seem to be definitely required in order to substantiate the feasibility of various proposed concepts, to permit a choice between alternative concepts and to integrate the most suitable system components into a working breadboard model.

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MICROSCOPIC SYSTEM FOR MARS STUDY PROGRAM

I. INTRODUCTION

This report describes the final results of a five-month feasibility study, Microscopic System for Mars Study Program, which has been carried out by the Electronics Division of General Mills, Inc. (GMI) under Contract No. 950123 with Jet Propulsion Laboratory (JPL). The period of performance extended from October 30, 1961 to March 31, 1962, with the provision of an additional one-month period for preparing this final report.

Although the contract only specifies a final summary report, we have taken the opportunity to add technical appendices which present the detailed results of the major study phases. These appendices contain most of the significant material which previously had only appeared in topical monthly progress reports, and which, it was believed, should be appended to this report for ready reference.

The intent of this study program has been 1) to survey a wide range of possible design concepts which might be applicable to the proposed microscopic life-detection system and 2) to recommend the best combination of optical, mechanical and electronic principles, methods and techniques which seem most feasible for devising the complete system.

The first two months of the study were devoted to a general background study of 1) environmental factors, 2) probable characteristics of Martian microorganisms, 3) soil sample collection and size reduction, 4) atmospheric and soil particle processing, 5) microscopic observation and detection, and 6) overall systems analysis. We attempted during this two-month period to avoid any specific orientation in studying various approaches to the problem of automatic, remote life-detection.

During the third month of the study our efforts were directed toward rigorously satisfying the specific component and system requirements as set forth in JPL Technical Dissertion SW-1980; this was a period of serious concern wherein some of the specific contractual requirements (such as providing a collection capability of up to 100 grams from all conceivable types of surfaces) were perhaps too strictly interpreted on our part. These points were clarified, however, at a project meeting held with JPL technical representatives on January 31 and February 1, 1962. At this time it was mutually agreed that the contractual specifications represented tentative guide-lines for the feasibility study and that the essential aim was to develop a microscope system which would successfully accomplish the general mission requirement, i.e., to transmit video-micrographs back to Earth of processed samples which had the best chance of containing recognizable biological specimens.

The final two months of this study were devoted to establishing the design parameters for a life-detection system which reflects our considered opinion regarding the best means by which to perform the basic mission. On the basis of convincing meteorological and biological arguments we have focused upon a pneumatic sampling technique for collecting natural aerosols from the atmosphere and aerosolizable particles from the Martian surface. We have established general design parameters for an inertial impactor which uses a motor-driven centrifugal blower to induce a flow of aerosols into the collector. We have developed adequate design guide-lines for developing a centrifuge unit for density fractionation (in order to effectively isolate the lighter-density and presumably biological particles). In addition, we have established theoretical bases (in certain aspects supported by preliminary experimentation) for three feasible types of concentration techniques. The outlook is very promising that the refined samples can be concentrated within small areas approximately equivalent to the microscope field-of-view. Further, the basic optical parameters for microscopic observation and detection have been, in most cases, clearly established.

On the basis of various system compromises, we are recommending a two-module design concept for the microscope: one module using fixed focus, visible light for observation of the larger-particle sample fraction; the other module comprising an auto-focus, U. V. microscope for observing the smaller particles.

Our studies have resulted, therefore, in the evolution of a unified system concept which would seem to merit further experimental investigation. Section V of this report outlines a recommended program for carrying out the various experimental phases considered essential in the preliminary design of the microscope system. A period of at least nine months would be recommended for experimentally confirming the design concepts for the individual components and for the overall system.

The list of project staff members who have been most actively involved in the important aspects of this study includes: R. Ginsberg - microscopy; V. W. Greene - microbiology; D. Lundgren - sample collection and processing; R. Peterson - environmental factors (and project scientist); D. Rotenberg - electrohydrodynamic particle collection; W. L. Torgeson - sample collection and processing.

II. SUMMARY OF INDIVIDUAL STUDY PHASES

It is our intention here to provide only a brief summary of each study phase; the individual appendices to this report contain extensive discussions of the major areas of investigation. This format is intended to provide a clear and concise treatment of the most significant study results, uninterrupted by the extensive development of important concepts or by the inclusion of interesting though less important details.

A. Martian Environmental Parameters

The basic astrophysical, atmospheric and surface parameters for Mars are sufficiently well-established to provide reasonable environmental design criteria for the microscope system. To a large extent, the environmental effects to be encountered on Mars do not exceed in severity those which may be found in certain regions on Earth or in its stratosphere, and certainly a large number of remote, automatic scientific and military systems have been successfully designed for operation in such extremely hostile portions of the terrestrial environment.

The lower Martian gravity (equal to 0.38 times the terrestrial value) is one of the most significant design parameters: an important advantage in terms of reducing the gravitational stresses on load-bearing components and of facilitating the airborne suspension and transport of particulate material; a possible disadvantage in terms of increasing the time required for gravitational settling of sample particles in processing fluids.

With the exception of CO_2, which has been detected spectroscopically, the other constituents of the Martian atmosphere are not directly known. The various indirect arguments are convincing, however, for an atmosphere predominantly comprising inert gases such as nitrogen (94 percent by volume) and argon (4 percent), with some 2 percent of CO_2 and little more than trace concentrations of O_2 and H_2O.

A surface pressure range of 70 to 100 mb seems to be a realistic estimate, as does an overall planetary range in surface temperature of +30° to -120°C. The diurnal range of surface temperature has been shown by radiometric measurements to approach 100°C in the Martian equatorial zone. Other important features of the Martian atmosphere include a relative sparseness of water vapor or ice crystal clouds and an absence of precipitation processes in the usual sense. Both theoretical and observational evidence support the concept of a planetary wind circulation rather similar in major respects to that on Earth, even though somewhat more weakly developed.

The Martian environment should not pose any insurmountable challenges in the design of a successful microscopic life-detection system. Although careful account must be taken of the lower gravity and pressure condition on the Martian surface, these factors can be suitably used to realize certain design advantages. Likewise, the rather extreme range of diurnal temperature variation may appear to present a formidable problem, but here again its regularity may be used to program the collection phase for the most suitable daily period. A very striking advantage of the Martian surface is its solidity and relative uniformity, e.g., no oceans or high, rugged mountain peaks. Also, we are reasonably certain that the dark regions offer the most opportune locations for detecting evidence of lower forms of plant life and microorganisms. Furthermore, if the scientific probe should land in a generally less favorable locale, the atmospheric circulation of Mars is capable of short-range transport of larger particles and debris by surface creep and saltation and long-range transport of smaller particles by airborne suspension. Thus, the general properties of the Martian environment offer few obvious disadvantages and many potential design advantages if they can be properly realized.

B. Inferred Properties of Martian Microorganisms

The assumption must be necessarily made that many of the basic properties of Martian microorganisms closely resemble those found on Earth. All living organisms that now exist (with the exception of viruses) exhibit the following basic characteristics: 1) a common chemical composition, 2) a common physical organization into cells, 3) common chemical activities, 4) capacity for growth, and 5) capacity to adjust to environmental changes.

In terms of chemical composition, therefore, it is to be assumed that Martian organisms are predomantly comprised of water, and that they also contain protein, nucleic acis, lipids and carbohydrates. In terms of physical organization, it may also be inferred that the size and shape of Martian organisms will fall within the terrestrial range of experience, e.g., that most microbes usually found as free living autotrophes and saprophytes will lie in the size range of 1 to 100 microns. Also, it may be assumed that Martian organisms have a similar capacity to produce detectable physicochemical changes in their environment. In addition, Martian microbes may be assumed to grow and to reproduce and that these phenomena are manifested by an increase in cellular protoplasm. Finally, it may be assumed that microorganisms on Mars can adapt to environmental changes and stresses (within certain limits), but that ultimately they will die and their complex biochemical structure will be degraded to simple oxidized elements.

A Mars Microscope system can be conceivably used to evaluate the following biological criteria: 1) spectrophotometric analysis of chemical composition, 2) presence of physically organized cells and cell masses with varying degrees of organizational complexity and function, 3) chemical changes which are optically demonstratable, 4) increase in cell numbers or mass by culturing, and 5) change in size and shape of cells and progeny under environmental stress.

The easiest characteristic to study with the Mars Microscope system would be the detection of organized particles in the size range of 1 to 50 microns. If one finds artifacts analogous to terrestrial cells, and especially clumps of these artifacts, a major criterion for the presence of Martian life will have been met. Of course, more sophisticated chemical, biological and spectrophotometric techniques should be used (to the extent which is practicable) to supplement the observation of physical organization.

Our studies of relative microbial concentrations in terrestrial soil and air have revealed that aerosol collection from the Martian atmosphere represents a feasible (and in other engineering aspects, attractive) approach to microbial sampling. On Earth, the viable contamination level per gram of solid matter and per inanimate particle is similar in both soil and air, i. e., one organism per 10^4 non-viable background particles.

C. Sample Collection

By sample collection is meant the initial acquisition, the transport to a collection device, and the final deposition of the sample either upon a surface or into a container. On the basis of various meteorological and biological considerations, we have concluded that an adequate and representative sample of the Martian biosphere can be most readily acquired in the form of natural atmospheric aerosols or aerosolized particles from loose surface material. Thus, our studies of sample collection techniques have focused upon pneumatic methods, which are simpler and inherently more reliable than mechanical methods which must react more vigorously with the environment by drilling, scraping, grinding, or digging the Martian surface. Moreover, our adoption of an aerosol sampling approach has in no way limited our choice of subsequent processing methods (which could be easily modified to handle samples acquired by other means).

The basic properties of Martian aerosols have been closely analyzed. When the effects of lower atmospheric density and lower gravity are considered, it is found that the settling velocities for aerosols in the size

range of interest are lower by a factor of 2, as compared to those on the Earth, thus making the Martian atmosphere a favorable medium for particle suspension and transport. Also, limited terrestrial data on the hitch-hiking characteristics of microbial material adhering to mineral particles has suggested that this important effect must be properly taken in account in the design of an aerosol collector. (If this effect could be ignored, size fractionation could be directly performed during the collection process.)

The possible types of aerosol particle collection systems which have been considered include: 1) inertial impaction, 2) filtration, 3) sedimentation, 4) electrostatic precipitation, and 5) thermal precipitation. Most of our interest has centered upon an inertial impactor which uses a motor-driven centrifugal blower to induce a flow of aerosols through a nozzle unit and to deposit the particulate matter upon a collection substrate. By proper design it is possible to achieve relatively sharp cutoff features, so that only those particles above a certain size and density will be collected on the substrate, and a further advantage is that the particles can be collected within a relatively small area. On the basis of a very conservative estimate of a combined efficiency of 10 percent for the motor-blower unit, an input power of 20 watts has been calculated for a unit capable of collecting on the order of one milligram of material in 30 minutes. (A higher efficiency than 10 percent is undoubtedly attainable in practice.) The input power can probably be reduced to 10 or possibly 5 watts; experimental studies will be required to confirm this possibility. Another possible method for providing the necessary flow of aerosols is the gas ejector pump, which would be especially attractive for a non-recycling sampling operation. Calculations show that the gas ejector pump could entrain a volume of 300 cubic feet of ambient air at a rate of 3 cfm, with an expenditure of one pound of primary gas.

A type of electrostatic precipitator, which makes use of a centrifugal blower and a high voltage power supply to collect aerosols, has also been seriously considered. Calculations have shown that the power requirement

could possibly be held to 2 watts for the blower and to less than one watt for the high voltage electrical charger. The use of filter collectors has also been considered but the difficulty in subsequent removal of particles is viewed as a decisive disadvantage.

D. Sample Processing

No matter how the sample is collected, the following phases of sample processing appear to be essential:

1) removal of the particles from the collector

2) disadhesion of the microorganisms from their mineral matrix

3) isolation of the light-density biological constituents by density fractionation

4) division of the biological particles into selective size fractions

5) concentration of each size fraction into an area approximately equivalent to the field-of-view diameter for microscopic observation

Other optional aspects of sample processing which are worthy of further experimental study include enrichment culturing and staining.

The need for Item (1) above is illustrated by the fact that a thin sticky coating is probably required to effectively retain the particles on the impactor collection stage. Item (2) is necessary on the basis of our knowledge that terrestrial microorganisms are "hitch-hikers", adhering to inert particles, and that some chemical or mechanical means is required to produce disadhesion of particle agglomerates. Items (1) and (2) represent feasible processing concepts, but will require laboratory experimentation to develop suitable techniques.

Density fractionation is definitely required as a means of removing the inert background particles which typically outnumber microorganisms in terrestrial soil and air samples by a factor of 10^4. The only feasible concept here seems to be the separation of lower-density microbes from higher-density mineral particles by centrifugation in a flotation liquid of intermediate density (i.e., $\rho \approx 1.25$). On the basis of realistic assumptions, a time period of some 30 minutes would be required for centrifugal density fractionation; a time interval some 4000 times longer would be required to achieve equally effective density separation by gravitational settling. Numerous potential flotation liquids have been chosen on the basis of physical and chemical criteria; the ultimate choice of the most suitable liquid will depend upon experimental studies.

Our studies have indicated that filtration (or micro-mesh sieving) is the only feasible method for carrying out effective size fractionation of particles suspended in a liquid medium. The use of one intermediate filtration step for size fractionation appears feasible. Serial filtration becomes increasingly unattractive as the number of filter stages increases since the recovery ratio of the smallest particles can be seriously reduced. A 10-micron pore size filter has been selected as the best compromise to collect out the larger 10 to 50-micron particles for low-power observation and to pass the 0.5 to 10-micron particles through for eventual highly concentrated collection on a surface suitable for high-power observation.

The concentration of the light-density microbial fraction into the smallest possible viewing areas is of critical importance in simplifying the microscope design. If the microbial particle samples can be concentrated into areas on the order of 10^3 to $10^5 \mu^2$, the necessity for horizontal microscopic scanning may be eliminated altogether. The three possible approaches to this problem are centrifugal impaction, micro-area filtration, and electrohydrodynamic precipitation.

The centrifugal impaction method involves centrifuging the microbial particle fraction in a liquid of lower density ($\rho \approx 0.8$) in order to concentrate the particulates within a viewing area at some outer portion of the centrifuge. One very attractive application of this concept would be to pass the small centrifuge arm through the microscope stage and at the completion of the spinning cycle, to stop the viewing surface in the microscopic viewing position.

The micro-area filtration concept most directly applies to the concentration of the larger size particles on the intermediate filter which is used for size fractionation. Biological specimens have already been concentrated in preliminary laboratory experiments within a Millipore filter surface of 340 microns diameter. A further reduction to a filter area of 180 microns diameter or less appears quite feasible. The adaption of micro-area filtration to concentrated collection of the smaller 0.5 to 10-micron particles may also be possible.

The use of electrohydrodynamic precipitation for concentrated particle collection has been successfully demonstrated for electrodes as small as 1 mm diameter. The essential elements of this method are to charge the particles, transport them through a dielectric liquid and precipitate them at the collector by the force exerted by the electric field. The particles can be removed by turning off the field to prepare the collector for subsequent collections. Further theoretical and experimental work is required to further reduce the collector area, establish the quantitative collection efficiency and determine the particle size classifications.

Chemical processing methods have been studied with a view toward enhancing the microscopic detection of viable microorganisms. Culturing techniques would improve the probability of detection by increasing the number of living cells and permitting the development of cell clumps and microstructures not always present in the testing phase. Possible approaches to enrichment culturing might involve the incorporation of nutrients in the impactor collecting surface or flotation medium, or using the Martian

mineral background itself as enrichment media. Staining of microbes to increase contrast against their background (for light microscopy) would provide observational advantages, but very extensive experimental development is apparently required.

E. Microscopic Observation and Detection

A competent biologist, employing the most careful techniques and using the finest quality optical instrumentation could manually meet the performance requirements of the Mars Microscope optical system. He could exercise various options regarding the basic microscope system, specimen slide preparation, scanning routine, and focus and centering adjustments. If he achieved adequate imagery, he might recognize the shape of the particle from previous experience; if not, he could resort to a micro-spectrophotometric analysis or culturing.

The basic functions which a biologist might perform can be denoted as detection, logic and control, and can be shown schematically as follows:

Logic in Photomicrography of a Microorganism

Detection	Control
	1. Scan the slide with stage controls and move the focusing adjustment through its range.
2. Detect the appearance of some brightness difference in the field-of-view.	
	3. Operate the focus adjustment.
4. Detect the in-focus condition.	
	5. Move the stage in the proper direction.
6. Detect the centered condition.	
7. Recognize the object as a particular microorganism from its shape and form.	
	8. Photograph the object.
9. No recognition.	
	10. Project image to entrance slit of spectrophotometer.

An automatic microscopic life-detection system will require many, if not all, of the functions listed previously. The "control" functions involve mechanical motions relatively easy to automate, although high accuracy demands careful design; the "detection" functions are more difficult to automate. For example, the automatic recognition of a microorganism by its shape or form is an extremely difficult if not unsolvable problem. On the other hand, automatic focus detection can be readily accomplished by state of the art instrumentation.

Automatic operation of the Mars Microscope optical system must be achieved with minimum size, weight, and power, with high reliability over extended periods of time and under varying environmental conditions. It has been necessary, therefore, to investigate not only mechanisms by which each of the necessary functions might be accomplished, but also various trade-off compromises. Appendix E discusses at some length the factors affecting the optical design and some specific instrumentation which might accomplish particular functions.

The optical design study has shown that it is theoretically possible, but highly impractical, to attempt to automate all of the functions which a biologist might wish to perform manually. A much more feasible approach is to isolate particles according to a few specific biological criteria, concentrate them in an area approximating the field-of-view of the microscope, use the magnifications which will provide the most information (with automatic focus for high power-high N. A.) and to take video-micrographs of the area on which the particles have been concentrated. In short, the "detection" functions must be kept to an absolute minimum and operation logic kept as simple as possible if a highly reliable, miniature microscopic observation and detection system is to be achieved. This philosophy will not provide a system which will cover all possibilities, but it will provide a high probability of success in the presence of the most probably occurring microorganisms.

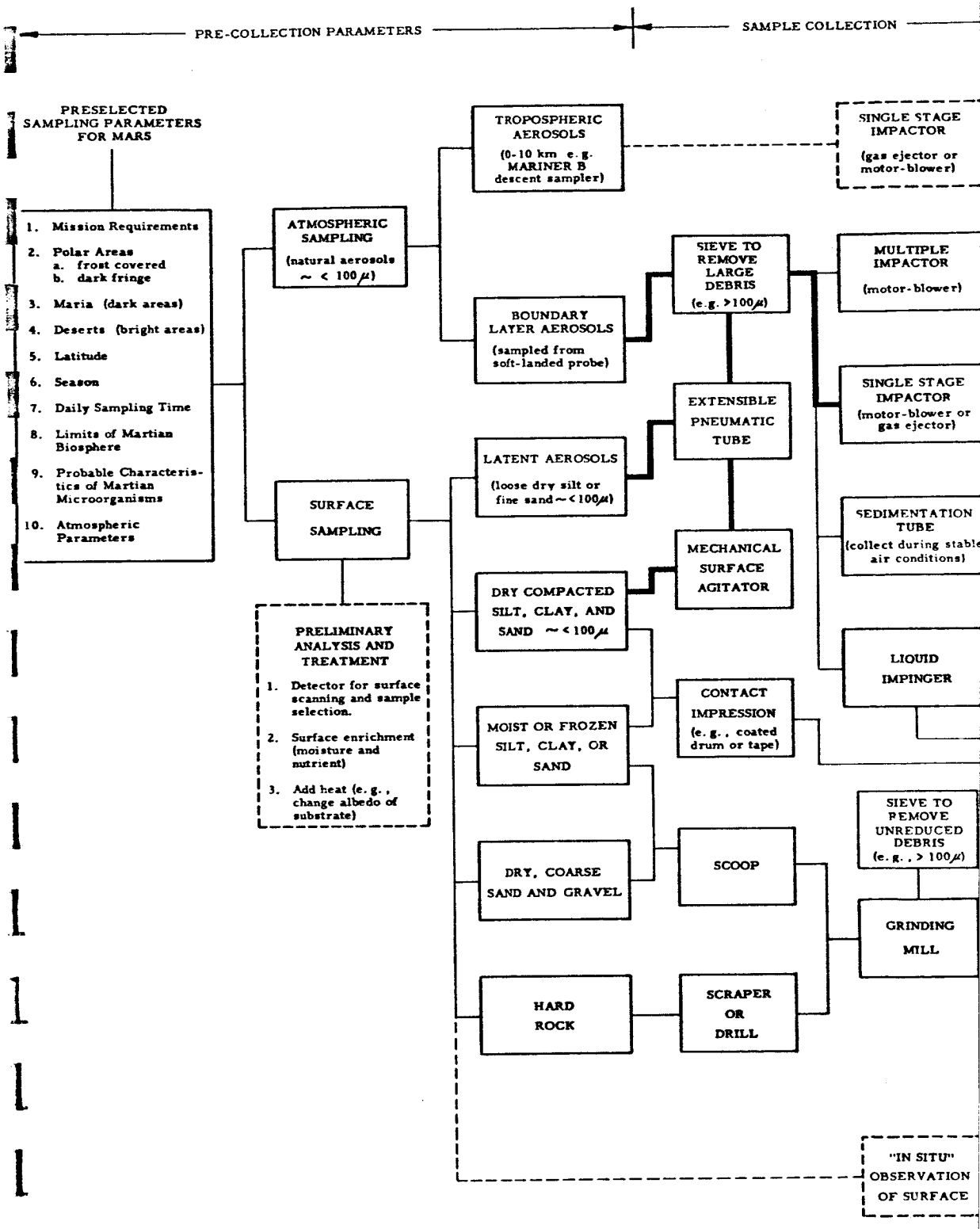
III. SYSTEM DESIGN CONCEPTS

The various instrumental components required for collecting, processing and observing the sample must be integrated into a harmoniously functioning unit which is compatible with the electronic subsystem. In spite of the necessarily complex sequence of operations which are entailed in a remote, automatic life-detection system, the combined sample handling and optical systems should preferably meet or at least not appreciably exceed the contractual specifications of 0.7 ft^3 for volume, 8 lb for weight, 2 watts continuous power consumption per operating cycle, and 20 watts peak power consumption for 5 minutes. Even though we have been advised in more recent discussions with JPL technical representatives that some relaxation may be possible in these basic specifications (by perhaps a factor of 2), they still represent desirable design objectives.

In comparing the advantages of competitive design concepts for individual components and their eventual integration into the overall system, a quantitative analysis of performance factors versus size, weight and power requirements is most desirable. To a certain extent, this type of quantitative analysis has been carried out during our studies whenever a realistic theoretical model could be developed. For example, in the case of inertial impactors for aerosol sample collection, the theory is sufficiently well-established (and is supported by empirical data) to permit a detailed analysis of aerosol collection efficiency in terms of parameters which describe the physical properties of the aerosols, the ambient air, the nozzle configuration and the flow rate. On the other hand, there are certain aspects of sample processing, such as density fractionation and specimen concentration, where overly refined numerical analysis would not produce meaningful results and where experimental studies will be ultimately required to obtain empirical performance data. Thus, our appraisal of feasible system design concepts has been necessarily based upon a combination of quantitative and qualitative analysis.

A detailed consideration of system design concepts can best be carried out with reference to Figure 1, which is a schematic flow chart outlining many of the possible design approaches to sample collection, processing, and observation and indicating their interrelationships. This chart is divided into five general sections: 1) pre-collection parameters, 2) sample collection, 3) sample processing, 4) observation and detection, and 5) electronics. (This last item has only been very sketchily indicated since this subsystem is JPL's responsibility.)

On the left-hand side of this chart have been listed several environmental parameters which would strongly influence the system design for a Mars life-detection mission. By carefully considering the effects of such parameters, we have analyzed the relative merits of atmospheric and surface sampling methods and have concluded that, on an overall basis, the sampling of boundary layer and latent aerosols represents the most feasible approach. Time did not permit a detailed comparison of mass sampling versus power consumption for all conceivable types of sample collection methods. And, indeed, if realistic theoretical models could be developed to analyze the relative collection efficiencies for sampling several representative types of ground surfaces (over the range from fine sand to hard rock), the ultimate design design decision would still have to depend upon some estimate of the probability of occurrence of such terrain conditions. Only the gross features of the Martian environment are presently known; the general appearance of the Martian surface is only defined to within a value of resolution some three orders of magnitude larger than the scale distance of the potential surface sampling area. On the other hand, the physical properties of Martian aerosol particles will be approximately the same, no matter where the instrument package should land, and they can be realistically estimated on the basis of the Martian density and gravity conditions. Since the calculated power consumption for a suitable aerosol sampling system falls within reasonable "ball park" range of the contractual specifications, and seems capable of significant reduction by experimental


PRE-COLLECTION PARAMETERS
SAMPLE COLLECTION
PRESELECTED SAMPLING PARAMETERS FOR MARS
1. Mission Requirements
2. Polar Areas
a. frost covered
b. dark fringe
3. Maria (dark areas)
4. Deserts (bright areas)
5. Latitude
6. Season
7. Daily Sampling Time
8. Limits of Martian Biosphere
9. Probable Characteristics of Martian Microorganisms
10. Atmospheric Parameters
ATMOSPHERIC SAMPLING
(natural aerosols ~ < 100 μ)
SURFACE SAMPLING
TROPOSPHERIC AEROSOLS
(0-10 km e.g. MARINER B descent sampler)
BOUNDARY LAYER AEROSOLS
(sampled from soft-landed probe)
LATENT AEROSOLS
(loose dry silt or fine sand ~ < 100 μ)
DRY COMPACTED SILT, CLAY, AND SAND ~ < 100 μ
MOIST OR FROZEN SILT, CLAY, OR SAND
DRY, COARSE SAND AND GRAVEL
HARD ROCK
PRELIMINARY ANALYSIS AND TREATMENT
1. Detector for surface scanning and sample selection.
2. Surface enrichment (moisture and nutrient)
3. Add heat (e.g., change albedo of substrate)
SINGLE STAGE IMPACTOR
(gas ejector or motor-blower)
SIEVE TO REMOVE LARGE DEBRIS
(e.g. >100 μ)
EXTENSIBLE PNEUMATIC TUBE
MECHANICAL SURFACE AGITATOR
CONTACT IMPRESSION
(e.g., coated drum or tape)
SCOOP
SCRAPER OR DRILL
MULTIPLE IMPACTOR
(motor-blower)
SINGLE STAGE IMPACTOR
(motor-blower or gas ejector)
SEDIMENTATION TUBE
(collect during stable air conditions)
LIQUID IMPINGER
SIEVE TO REMOVE UNREDUCED DEBRIS
(e.g., > 100 μ)
GRINDING MILL
"IN SITU" OBSERVATION OF SURFACE

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SAMPLE PROCESSING

COLLECT PARTICLES LARGER THAN CUT-OFF SIZE DIRECTLY ON MICROSCOPE SLIDE

TRANSPORT SLIDE TO MICROSCOPE (if necessary)

DISSOLVE COLLECTION SUBSTRATE TO RELEASE PARTICLES

LIQUID TRANSPORT OF SEPARATE SIZE FRACTIONS TO FLOTATION CHAMBER

CONCENTRATED COLLECTION OF SMALLER PARTICLES BY EHD PRECIPITATION

EHD DEVICE INTEGRATED INTO MICROSCOPE STAGE

CONCENTRATED COLLECTION OF SMALLER PARTICLES BY CENTRIFUGAL IMPACTION IN LIQUID OF $f=0.8$

PARTICLE COLLECTOR ON CENTRIFUGE STOPS AT MICROSCOPE STAGE

LIQUID TRANSPORT TO FLOTATION CHAMBER

FILL CHAMBER WITH FLOTATION LIQUID ($f \approx 1.2$)

CONCENTRATED COLLECTION OF SMALLER PARTICLES BY MICRO-AREA FILTRATION

TRANSPORT SLIDES AND/OR FILTERS TO MICROSCOPE STAGE

INTERMEDIATE SIZE FRACTIONATION BY FILTRATION

CONCENTRATE LARGER PARTICLES ON FILTER

SEPARATE MICROBES FROM MINERAL MATRIX (agitation or detergent)

FLUID OR MECHANICAL TRANSPORT TO FLOTATION CHAMBER

COLLECT SIZE FRACTIONS ON SEPARATE SLIDES

LIQUID TRANSPORT TO FILTER OR SLIDE COLLECTORS

SIZE FRACTIONATION BY MULTIPLE IMPACTION

DENSITY FRACTIONATION BY CENTRIFUGATION

REMOVAL OF SUPERNATE (e.g., centrifugal valve & plenum)

AEROSOLIZE SUPERNATE

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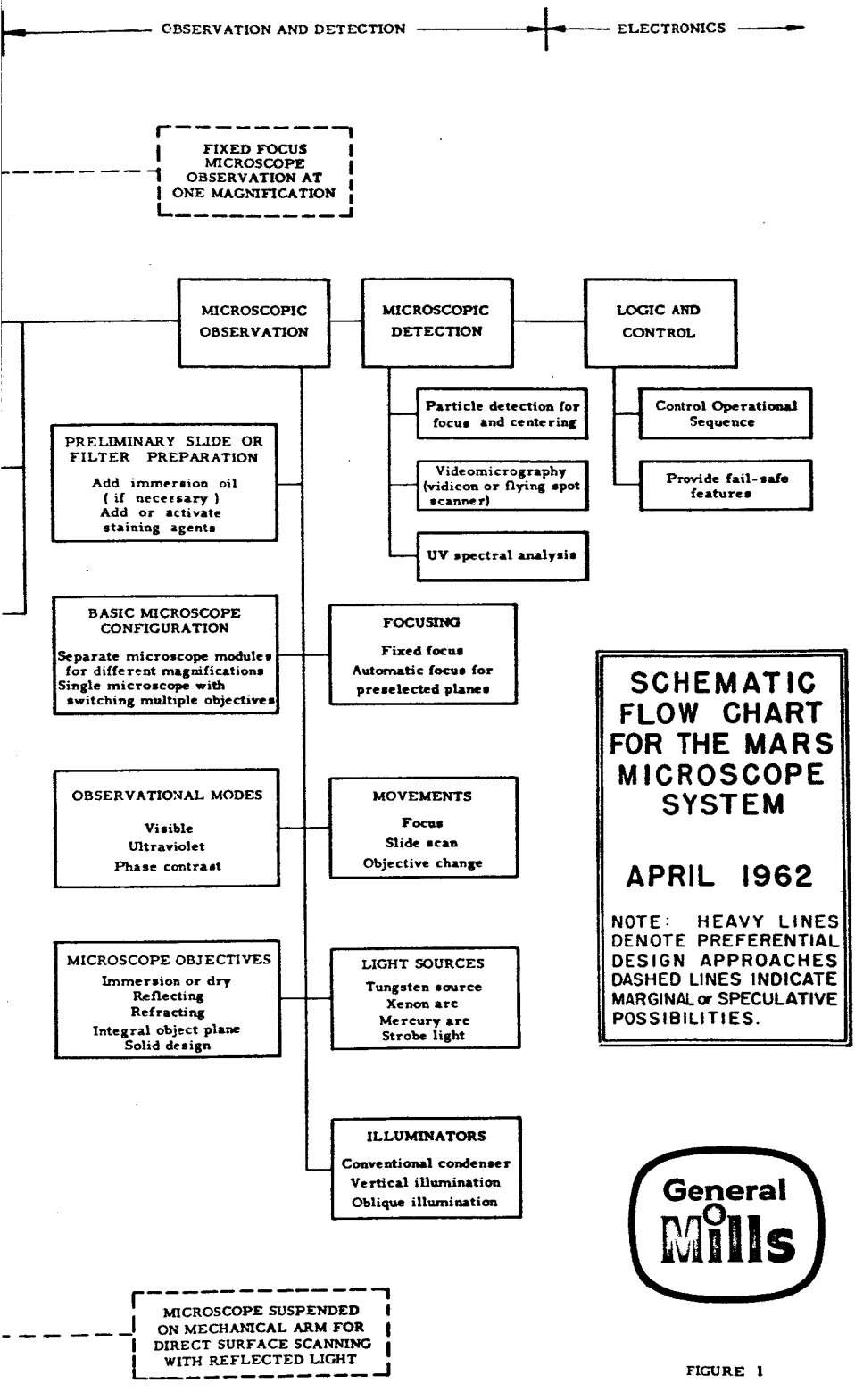

SCHEMATIC FLOW CHART FOR THE MARS MICROSCOPE SYSTEM

APRIL 1962

NOTE: HEAVY LINES DENOTE PREFERENTIAL DESIGN APPROACHES DASHED LINES INDICATE MARGINAL or SPECULATIVE POSSIBILITIES.

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FIGURE 1

design studies, we have felt quite justified in adopting the aerosol sampling approach (which also provides the advantage of inherently higher reliability since it does not mechanically interact with an essentially unknown surface environment).

Four basic types of aerosol collection systems have been indicated on the flow chart (although there are many other possibilities, including the electrostatic precipitator which is discussed in Appendix C of this report). Our studies have pointed to the single stage impactor as the most feasible type of aerosol collection; a motor-driven centrifugal blower would be used to induce the flow of aerosols into the impactor. The additional complexity of the multiple-stage impactor affords no compensating advantage for our application since our other studies have shown that microbes should first be separated from particle agglomerates prior to size fractionation. The liquid impinger affords a considerable advantage in terms of collecting the particles on the surface of the processing liquid, but there is a more important offsetting disadvantage in exposing the processing liquid to outside ambient conditions during the collection phase. By means of an extensible pneumatic tube, the inertial impactor collector can be readily adapted to sample loose surface particles. A simple sieve can be designed into the inlet to prevent the entry of large debris, without seriously compromising the performance characteristics. As stated earlier in this section, the collection efficiency of inertial impactors is amenable to detailed mathematical treatment, so that we can calculate with some certainty the minimum particle size cutoffs for an impactor of specific design. Further, we can establish the trade-off characteristics for cutoff size versus flow rate and power input. The power consumption for a combined motor-blower unit can be estimated on the basis of assumed values for the electrical efficiency of the drive motor and the adiabatic efficiency of the blower; our estimate of 10 percent for combined efficiency is probably too conservative, so that our estimate of 20 watts for the power consumption of a representative inertial impactor may be too high by a factor of 2. A program of experimental

studies with model impactor units will definitely be required to establish firmer estimates of power consumption for a given level of performance.

The schematic flow chart also clearly outlines the recommended processing routine for refining the aerosol particles: 1) dissolve the collection substrate to release the particles into solution, 2) transport the particles in liquid suspension to the flotation chamber, 3) fill the chamber with flotation fluid to the required level, 4) separate the microbes from the adherent mineral particle matrix by mechanical or chemical action, 5) perform density fractionation by centrifugation, 6) remove the supernate of light-density (presumably biological) particles by simple fluid mechanical means, 7) transport the supernate to the filter or slide specimen collector, 8) perform one-stage size fractionation by filtration (and in the same process concentrate the larger 10 to 50-micron particles upon a small filter surface which is mechanically transported to the low-power microscope module), and 9) concentrate the smaller 0.5 to 10-micron particles by centrifugal impaction, micro-area filtration or electrohydrodynamic precipitation upon a viewing surface appropriate for observation by the high-power microscope module. We have indicated on the flow chart some possibilities for eliminating the transport of the smaller-particle specimens wherever the collector units can be integrated into the microscope design. One possibility would be to incorporate the electrohydrodynamic collector directly into the stage of the high-power microscope, as illustrated in Figure 2 (with its associated explanation of component code numbers in Table 1) and in Figure 3, a detail diagram of the condenser stage and objective portion of the high-power microscope. Another approach would be to design the collector unit on the centrifugal impactor to pass between the condenser and objective of the high-power microscope during the spinning cycle (using an auto-focus mechanism to temporarily increase the clearance tolerance) and to stop the collector unit at the proper stage position.

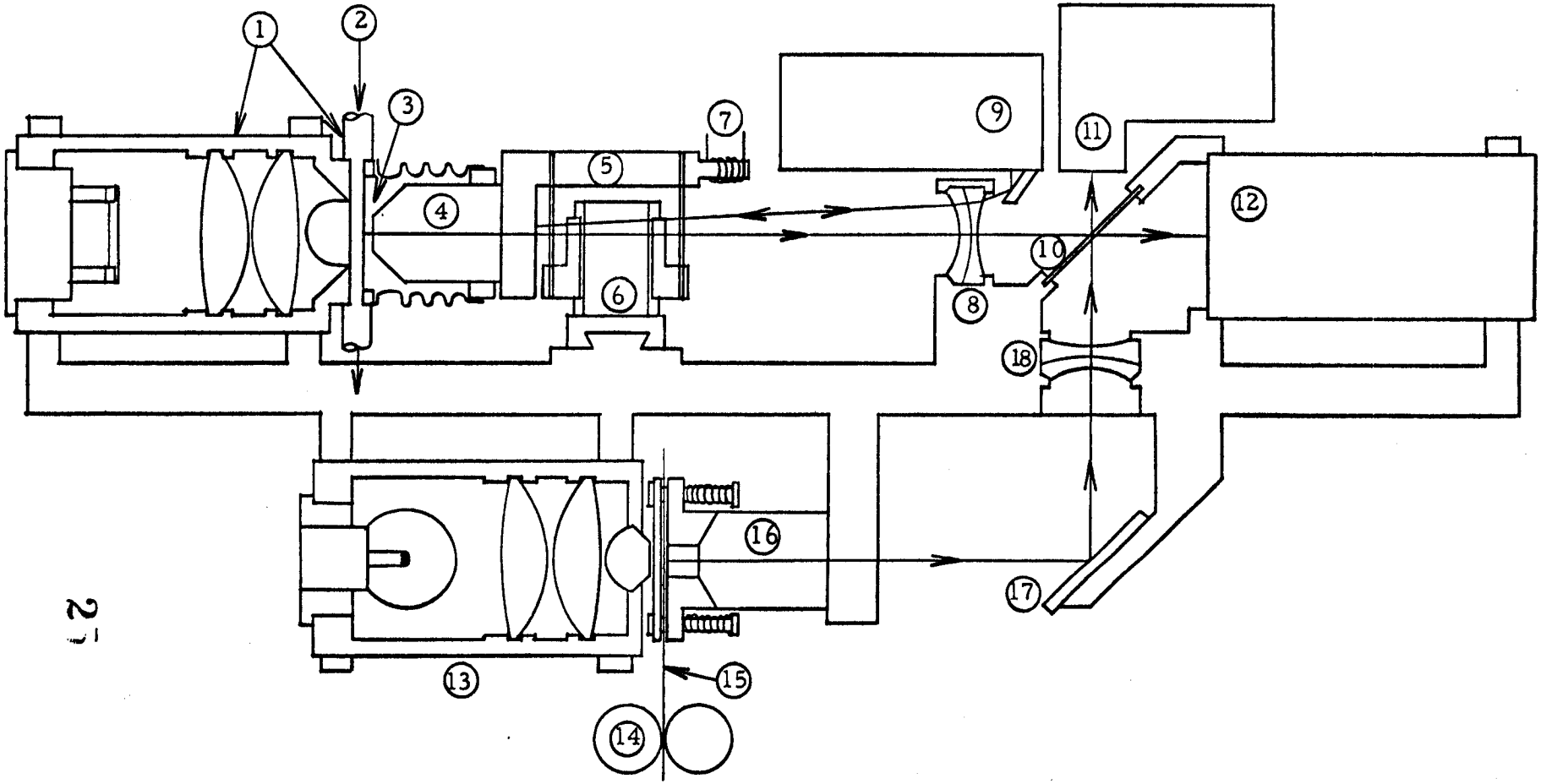

Figure 2. High and Low Power Microscope (Schematic)
(See Table 1 for explanation of component code numbers)

Table 1. Key to Schematic for High and Low Power Microscope

1. U. V. Illuminator - condenser with attached sample chamber
2. Particles in fluid from processing equipment
3. Immersion fluid chamber in flexible enclosure
4. High power U. V. objective
5. Fine focusing motion
6. Field scanning motions
7. Fine focusing drive
8. Amplifier lens for high power objective
9. Automatic focus optics
10. Beam splitter (or beam splitter for high power and mirror for low power)
11. Particle detector (scanning photometer or spectrophotometer)
12. Vidicon
13. Visible light illuminator and condenser
14. Filter tape transport mechanism
15. Filter material on a tape base
16. Low power objective with integral object plane
17. Relay mirror for low power objective
18. Amplifier lens for low power objective

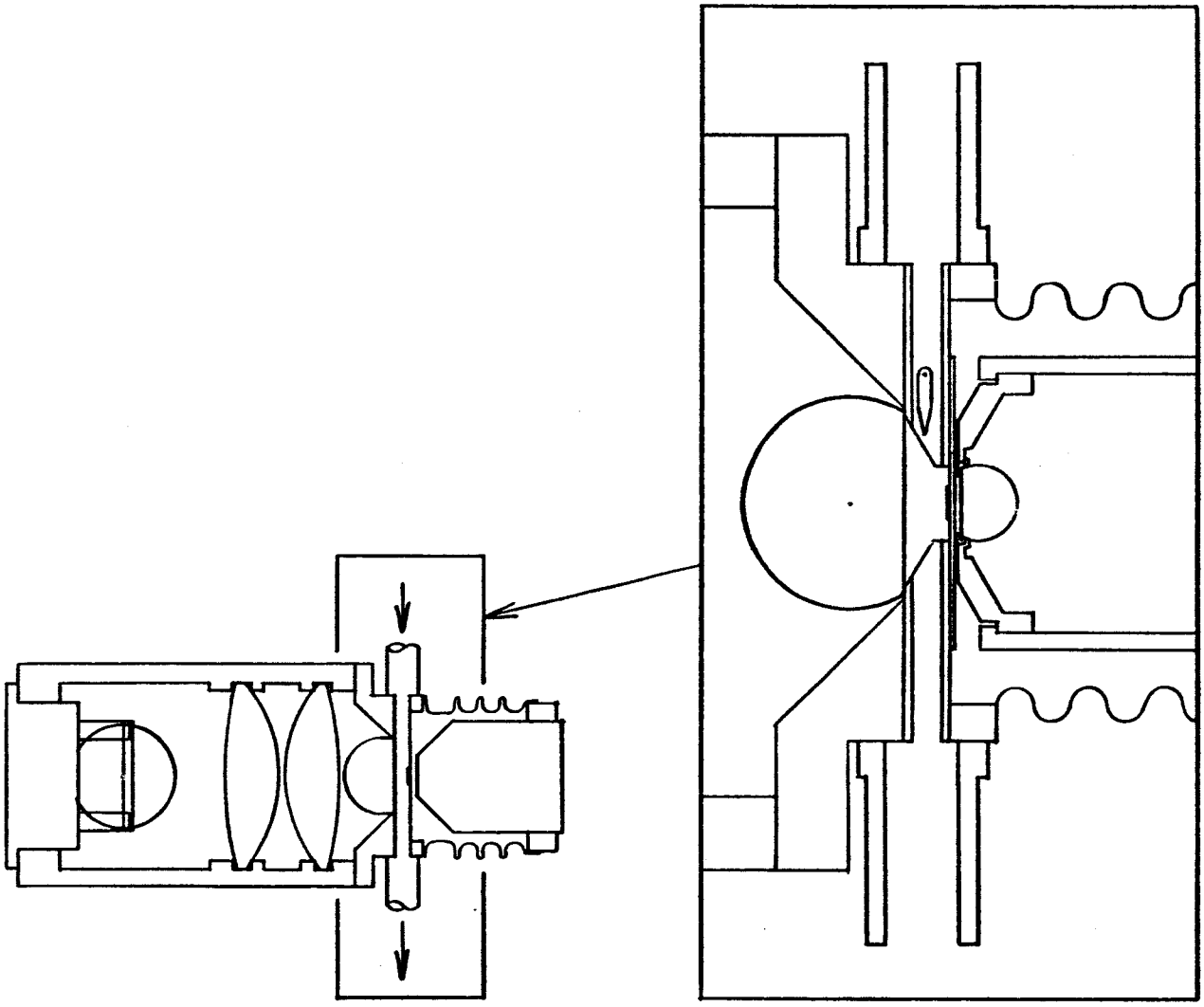

Figure 3. Detail of Fixed Sample Chamber Particle Concentration by E. H. D. (Schematic)

There are, of course, numerous possible design trade-off areas involved in the sample processing routine. One very basic design problem arises in connection with the specification that the microscope system be capable of repeating at least twenty-five cycles for the complete mission. The approach which we have adopted is to use an expendable collection and processing module for each observation. This would imply a series of miniature units, one of which would be brought into position beneath the impactor nozzle before the start of each collection cycle. Each unit would contain a collecting substrate, a miniature centrifuge chamber, a self-contained supply of flotation fluid, and other basic components such as centrifugal valve and plenum for removing the supernate after centrifugation, and other optional components such as the centrifugal impaction device (a secondary centrifuge with a self-contained supply of lower-density fluid for concentrated specimen collection). There are, of course, serious design implications with this modular approach in terms of providing engagement and release devices and in overall terms of size and weight requirements. On the other hand, if reliable fail-safe features can be provided, the modular design affords desirable redundancy in the event of an internal failure within a particular processing unit and further, it eliminates the need for flushing out the detritus from earlier processed samples before each operation. It should also be mentioned that the same motor used to drive the blower for the aerosol collector would be employed to perform the centrifugal processing functions.

In the section of the flow chart which describes observation and detection, we have tried to indicate the more important aspects of optical design which are involved. Appendix E to this report contains a detailed discussion of the various optical trade-offs which can be made in designing the microscope subsystem (of particular importance is the degradation of resolution as a function of increasing out-of-focus condition). In addition, the relative merits of light and U. V. microscopes are thoroughly discussed: U. V. microscopy may not be justified in terms of improved resolution quality

alone but is attractive, however, as a means for providing possible micro-spectrophotometric U. V. absorption analysis. Our adoption of a modular design concept for separate low- and high-power observation, as shown in Figure 2, has been with the realization that some additional size and weight penalties are involved; nevertheless, if each microscope unit can function independently, it would not be adversely affected by the temporary malfunction or permanent breakdown of the other unit. In addition, the simplification of the low-power microscope design to a fixed-focus design with no objective switching or horizontal specimen scanning should not only markedly increase its reliability, but also should correspondingly increase the reliability of the high-power microscope.

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IV. CONCLUSIONS REGARDING A FEASIBLE MICROSCOPE SYSTEM

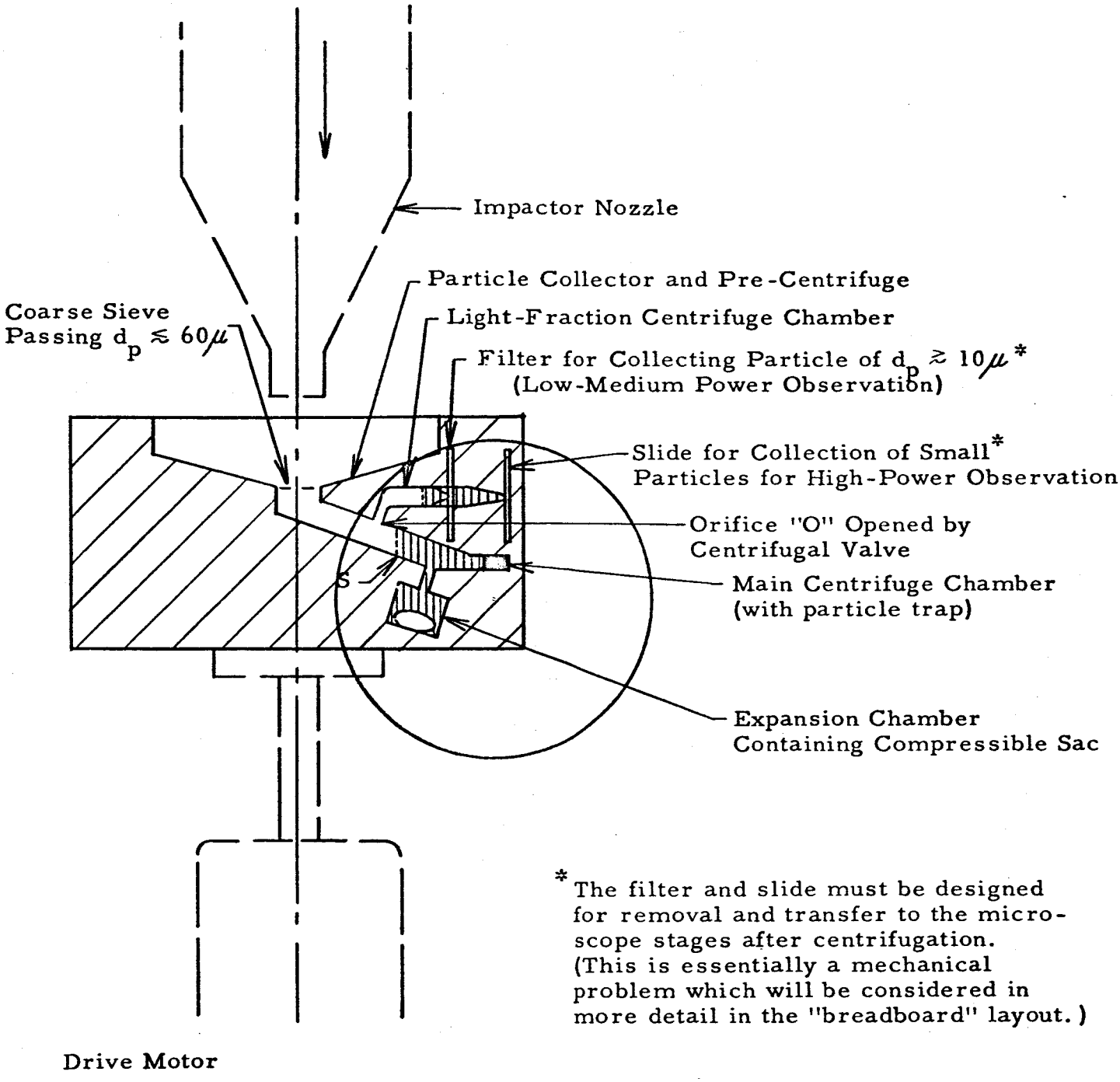
It should be stressed that our conclusions regarding a feasible design concept for the microscope system are based upon the results of a five-month study program; in no sense can these conclusions be regarded as definitive without more experimental and theoretical evidence. Nevertheless, an overall system design concept has resulted from our study which shows great promise for fulfilling the basic mission requirement: the remote, automatic microscopic observation of refined particle samples from the Martian environment to detect the presence of possible life-forms. It should be pointed out, however, that there are design alternatives for certain of the system components, and that an extensive program of experimental studies would be required in order to make the best choice. In addition, there are certain optional components, such as microspectrophotometric devices for U.V. absorption analysis, which appear to be quite desirable from the point of view of providing supporting evidence for the presence of biological constituents, but which are not strictly necessary in terms of the basic mission requirement to transmit video-micrographs of selected particle specimens.

If we now consider our detailed conclusions regarding feasible design parameters for the individual system components, the most promising approach to sample collection is inertial impaction of atmospheric aerosols and of aerosolizable particles from the Martian surface. We have concluded that a milligram sample of such particles per observational cycle is within the feasible range for insuring an adequate biological fraction (on the basis of microbial concentrations which are typical of barren terrestrial regions). The term "adequate" is used in the context here of highly refined specimens which are highly concentrated within a large portion of the microscopic field-of-view. We have also concluded that a period of 30 minutes would be required for sample collection in the case of atmospheric aerosols and could be considerably less where high local concentrations of aerosolizable particles are present on the underlying ground surface.

We have concluded that a highly efficient method for density fractionation constitutes the most critical processing requirement. Mineral particles outnumber biological particles in terrestrial surface and air samples by a typical factor of 10^4. The mineral particles must be removed, therefore, with a very high degree of efficiency. Density fractionation in a flotation liquid whose density is intermediate between the lighter density microbes and the higher density mineral particles is currently regarded as the only feasible approach. Furthermore, we have concluded that centrifugation is the only practical method for achieving effective, rapid density fractionation; by comparison, gravitational sedimentation is slower by a factor of 4×10^3. There are other subsidiary problems involved in the density fractionation process, such as the reduction of microbial-mineral particle agglomerates, which appear to be amenable to solution by mechanical or chemical techniques. We have also concluded that an expendable module concept is most applicable for performing density fractionation (and for fulfilling other processing requirements); otherwise, the centrifuge chamber would have to be flushed following each operational cycle.

The next important step in the processing routine is the removal from the centrifuge chamber of the supernate which contains the lighter density (and presumably biological) particles. The simple fluid mechanical method we propose for accomplishing this supernate removal is shown in Figure 4. It essentially involves a centrifugal valve and compressible sac which are activated by changes in the rotational speed of the centrifuge and result in driving the surface layer of the supernate into an adjacent chamber. The simplicity of this design concept makes it quite appealing.

Another major conclusion of our study is that great emphasis must be placed upon the development of methods for highly concentrated collection of refined specimens on a suitable viewing surface. Here, three possible design approaches have been developed during the course of this study: 1) centrifugal impaction, 2) micro-area filtration, and 3) electrohydrodynamic precipitation. (These methods are discussed at some length in



* The filter and slide must be designed for removal and transfer to the microscope stages after centrifugation. (This is essentially a mechanical problem which will be considered in more detail in the "breadboard" layout.)

Figure 4. Schematic of Differential Centrifugation Device for Sample Processing

Appendix D to this report.) There are important design implications inherent in each method. In the case of centrifugal impaction, another liquid is introduced into the system which is of lighter density than the "biological" particles, and the particles are collected by centrifugation within the highly concentrated volume of a special viewing device on the outer wall of the centrifuge chamber. This special viewing device might be designed to stop at the microscope stage when the spinning cycle is completed. In the case of electrohydrodynamic precipitation, concentrated collection of the refined particles might be made upon a small electrode which is integrated into the design of the microscope stage; the particles would be brought to the microscope stage in a dielectric liqud, temporarily collected on the stage for observation, and eventually flushed away. Further experimental studies will be definitely required to analyze the relative merits of these alternative particle concentration methods.

Our studies of size fractionation techniques have concluded that filtration (or micro-mesh sieving) is a feasible approach to this aspect of particle processing. We have also concluded that this filtration method can best be used to fractionate the refined samples into two size ranges: approximagely 0.5 to 10 microns, and 10 to 50 microns. A more extensive breakdown into several size fractions by serial filtration would be possible but would require a more complex unit to transport the various filter specimens to the microscope and would result in appreciable particle losses if smaller pore-size filters were used. A 10-micron pore-size filter represents a desirable design compromise, since, as will be subsequently shown, the resulting size fractions are quite suitable for microscopic observation by separate low- and high-power microscope units.

On the basis of optical considerations, as well as overall system requirements, we have concluded that a two-module microscope represents the best design approach for our application. The low-power unit, whose basic parameters are outlined in Table 2, would be a simple fixed-focus, fixed-field, visible light, 100X microscope for observing the larger 10 to

Table 2. Low Power Microscope Parameters

Visible Transmitted Light -- for particles 10-50μ diameter

Fixed Focus -- 15μ from slide

Fixed Field -- (no scan)

No Particle Detection -- take photos at preselected times

Sample Holder -- Filter material

Sample Area -- 350μ or less

Objective -- 10 X 0.25 N. A.

Amplifier -- 10 X

Overall Magnification -- 100 X

Field-of-View -- 180μ

Resolution -- 1.1μ (theoretical)

Depth of Field -- 8.4μ $\quad d_{max} = 4.2\mu$, after that $z = \frac{d}{4}$

Distance from Slide	Resolution
11μ to 19μ	1.1μ (theoretical)
7μ , 23μ	2μ
3μ , 27μ	3μ

Alternatives

1) Incident dark field or oblique illumination

2) U. V. instead of Visible Light

50-micron particles collected on filters. The basic parameters for the high-power unit are presented in Table 3. This more complex U. V. immersion, 1000X microscope would be used for the observation (and detection) of the smaller particles below 10 microns diameter. Provision has thus been made in the high-power ultraviolet microscope for the possible inclusion of a microspectrophotometric device to analyze the differential absorption characteristics of biological particles. We have also indicated in Table 3 one of the possible design choices for the automatic focusing device; in this instance, three pre-selected focus positions would be used at 0.5, 1.0 and 2.0 microns from the slide. This type of focusing device would be simpler but would result in compromized resolution qualities as compared with a complete range of focus capability.

In summary, therefore, we have concluded that a feasible design concept can be developed for a microscope system which is capable of fulfilling the basic mission requirement. By suitable integration of well-established and new design concepts for each of the components, a relatively simple system appears to be feasible for carrying out the required functions for sample collection, processing and microscopic observation. An extensive program of experimental studies would now seem to be necessary to establish the performance characteristics of individual components, to make the best choice between alternate design concepts, and to determine the design parameters and for the integrated system.

Table 3. High Power Microscope Parameters

U. V. Immersion -- for particles below 10μ diameter

Automatic Focusing -- 3 position; e.g., 0.5μ, 1.0μ, 2.0μ from slide with accuracy = 0.1μ. Detection of structure triggers photo.

Sample Holder - Fixed E. H. D. Chamber -- fluid sample transport

Sample Area - 100μ diameter or less

Scanning System -- to cover sample area

Particle Detector -- detects structure or differential absorption

Objective -- Zeiss 100 X 1.25 N.A. Glycerin Immersion

Amplifier -- 10 X

Overall Magnification -- 1000 X

Field-of-View -- 20μ diameter

Resolution -- 0.15μ (theoretical)

Depth of Field -- 0.19μ

Alternatives

1) Concentration by centrifugal impaction upon specially designed slide; slide brought into microscope field-of-view without removal from centrifuge assembly.

2) Focus over desired range, with particle detector choosing focus setting for best photo.

3) Fixed field with sample area of 50μ diameter or less (no scan, no detection; photos at selected focus settings).

4) Sample collected on filter material.

V. A RECOMMENDED EXPERIMENTAL STUDY PROGRAM

The results of our study have produced an overall system design concept which seems feasible on the basis of theoretical arguments and limited experimentation. Most of the potential problem areas associated with the collection, processing, and observation of Martian air and soil samples have been identified. There are, however, some important aspects of the component and system design which cannot be satisfactorily resolved without more extensive experimentation. In the area of sample concentration, for example, there are three feasible concepts whose comparative merits can only be adequately evaluated by laboratory tests with working models. The following outline summarizes the various phases which are recommended for inclusion in a follow-on program of experimental studies.

1) Biological Support Study

   a) Preparation of simulated Martian biomixture and inert background (to provide realistic samples for evaluating collection, processing and observation techniques).

   b) Microscopic observations of biomixture (to study the observational interference from mineral particles for samples in various states of purification).

2) Sample Collection

   a) Generation of aerosol flow into collector (to determine most efficient method for inducing a flow of natural aerosols).

   b) Aerosolization of particles from soil surfaces (to establish the design parameters for aerosolizing surface particles).

   c) Impactor configuration (to determine best compromise between collection efficiency and power requirement).

d) Collection substrate (to analyze the collection and retention characteristics of various materials which are readily dissolvable and biologically compatible).

e) Integrated collection system (to establish the design parameters for the overall system).

3) Preliminary Processing

a) Removal of particles from collection substrate (to determine the most effective dissolving liquid and method of activation).

b) Reduction of particle agglomerates (to evolve the most efficient method to separate biological particles from their mineral micro-matrix).

c) Removal of extra-large particles (to establish best sieving method to remove particles whose size would interfere with subsequent processing).

4) Density Fractionation

a) Flotation liquid (to determine most suitable liquid, minimum volume requirement, storage method, activation and environmental criteria).

b) Centrifuge chamber - size and configuration.

c) Centrifuge chamber - operational conditions (to determine most efficient program for density fractionation as function of centrifugal speed and operation time).

d) Removal of light-density supernate from centrifuge (to evolve an effective fluid mechanical system for removing the supernate from the flotation liquid).

5) Size Fractionation

a) Basic studies of serial filtration (to determine the extent to which sub-pore size particles are lost during passage through a series of filters).

b) Integration with concentration techniques (to incorporate the filters for size fractionation into the device used for concentrating the final specimen on a viewing surface).

6) Concentration

a) Centrifugal impaction (to determine the effectiveness of particle concentration by centrifugation in a liquid of relatively lower density, i.e., $\rho \approx 0.8$).

b) Micro-area filtration (to determine the smallest filter area which can be effectively used for concentrating particles).

c) Electrohydrodynamic precipitation (to determine quantitatively the percentage of particles recoverable from various liquid suspensions by using electrohydrodynamic techniques).

7) Observation and Detection

a) Low-power observation (to determine the optimum microscopic method for observing larger microbial particles collected on sieves, filters and other materials).

b) High-power observation (to analyze the observational characteristics of smaller microbes on different surfaces by using transmitted light, phase contrast and possibly U.V.).

c) Detection (to evaluate the potential usefulness of various photometric techniques for particle detection).

8) Overall System Considerations

a) Special miniaturization problems.

b) Electromechanical actuation, switching and control devices.

c) Environmental control requirements, e.g., temperature.

d) Weight, size and power requirements.

e) Reliability factors (including "fail-safe" design provisions).

f) Operational programming requirements.

g) Final breadboard design.

APPENDIX A

MARTIAN ENVIRONMENTAL PARAMETERS

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I. INTRODUCTION

The design of an automatic microscopic life-detection system which will operate effectively in the Martian environment obviously depends upon an informed estimate of significant astrophysical, atmospheric and surface parameters. Even though relatively few of these key parameters have been definitively measured, it is possible to establish realistic limits for the environmental factors which will conceivably affect the system design.

Especially within the past decade, an extensive literature has evolved from the visual and photographic, spectrophotometric, polarimetric and colorimetric observations of Mars, and from the theoretical speculations of astronomers, astrogeophysicists, exobiologists and others. The bibliography in Appendix F to this report includes many of the important literature sources which were examined during this study. However, it is not our intent to present here an extensive critical review of this material; recent articles by de Vaucoleurs (1960) and Hess (1961) contain excellent summaries of observational and theoretical work on this subject, and, of course, de Vaucouleurs' book, Physics of the Planet Mars (1954), is the classic compendium of detailed areophysical information. To a very large extent, the following discussions have been based upon material appearing in these latter references.

II. ASTROPHYSICAL PARAMETERS

Pertinent astrophysical data for Mars are shown in Table A-1 (based upon a combination of values recently presented by de Vaucoleurs and Hess). As a result of its almost identical rotation rate and similar inclination of equator to orbital plane, Mars closely resembles the Earth in terms of its length of day and in the marked seasonal differences in the distribution of solar radiation received over its surface. The Martian year is, however, nearly twice as long, and in addition, the greater eccentricity of

Table A-1. Astrophysical Data for Mars

(from de Vaucouleurs 1960 and Hess 1961)

Element	Absolute Units	Units Relative to Earth
Mean distance from sun	228×10^6 km	1.524
Perihelic distance	206×10^6 km	-----
Aphelic distance	248×10^6 km	-----
Eccentricity of orbit	0.0933	5.5
Inclination of equator to orbital plane	25°	1.06
Sidereal revolution period	687 days	1.881
Duration of seasons in northern hemisphere		
(1) Spring	199 days	-----
(2) Summer	182 days	-----
(3) Autumn	146 days	-----
(4) Winter	160 days	-----
Synodic period (time interval between oppositions)	780 days	-----
Earth-Mars distance at optimal opposition	56×10^6 km	-----
Period between optimal oppositions	Approximately 15 years	-----
Length of day (solar time units)	24 h 37 m 22.7 s	1.0012
Mean diameter	6739 km	0.529
Mass	----	0.107
Volume	----	0.150
Surface gravity at 45° lat.	377 cm sec^{-2}	0.384
Escape velocity	5.0 km/sec	-----
Visual Albedo	0.15	0.43

the Martian orbit is associated with seasons of more unequal length. The synodic period (or time interval between oppositions, when the Earth lies most nearly between the Sun and Mars) is approximately 25-1/2 months. The Earth-Mars distance at opposition varies from 56×10^6 to 100×10^6 km, with the optimal opposition occurring at 15-year intervals (or every seventh opposition).

Because of its smaller planetary mass and size, the surface acceleration of gravity on Mars is slightly more than one-third of the terrestrial value. This lower gravity has a marked effect upon the gases which can be retained in the Martian atmosphere and produces a correspondingly smaller value of adiabatic, vertical lapse rate of temperature.

As a result of its relatively low planetary albedo and high atmospheric transmission coefficient, the effective available energy absorbed by the Martian surface (as compared to that on Earth) is considerably more than might at first be suggested by its greater distance from the Sun. For example, at latitude 45 degrees, the average intensity of solar radiation received at the Martian surface is about 75 percent of the corresponding terrestrial value (at the poles and the equator, the comparable values are about 110 and 65 percent, respectively). As a result, the average planetary temperature of Mars, although lower than the Earth's, is not as low as it would otherwise be except for the effects of the albedo and atmospheric transmission factors.

III. ATMOSPHERIC PARAMETERS

A. Composition

With the exception of a few reports which could not be verified independently, the only positively identified gas in the Martian atmosphere is CO_2. The near-infrared absorption bands of this gas near 1.6 and 2.0 microns were detected directly by Kuiper in 1947-1948. Recent theoretical

calculations by Goody and Grandjean have concluded that Mars may have close to thirteen times the terrestrial content of gaseous CO_2.

The absorption spectrum of oxygen has not as yet been detected in spite of numerous attempts. On the basis of instrumental sensitivity, Dunham (1949) has calculated that there is probably not more than 0.0015 as much oxygen per unit area on Mars as on Earth. Similar efforts to detect water vapor have also produced negative results. Hess has concluded that no more than an indeterminate but very small amount of vapor can be present in the Martian atmosphere; he has presented an extreme upper limit of about 0.1 mm of liquid water equivalent, or approximately 10^{-2} g cm^{-2}. The most convincing argument for some water vapor being present in the Martian atmosphere (probably occurring mostly in ice crystal form) is the fact that the polar caps are almost surely composed of H_2O.

On the basis of indirect arguments based upon the theory of escape of planetary atmospheres, cosmic abundance and chemical properties, it has been generally agreed that nitrogen (whose ultraviolet absorption bands are unfortunately indetectable by observations through the terrestrial atmosphere) is the most likely major constituent of the Martian atmosphere, accounting for perhaps 94 percent by volume. On the basis of the assumption that the production of argon as a decay product of radioactive potassium has been similar to that on Earth, it has been proposed that some four percent by volume of the Martian atmosphere may consist of argon.

As de Vaucouleurs has recently stated it, "Indirect methods such as chemical theory and dynamical theory eliminate as plausible constituents most gases except CO_2 + CO, N_2, H_2O, O_2 + O_3, and the rare gases". The most commonly accepted estimates for the atmospheric composition of Mars are: N_2, 93.8 percent by volume; A, 4.0 percent; CO_2, 2.2 percent; O_2, < 0.1 percent; and H_2O, < 0.1 percent (with perhaps as much as an order of magnitude in its seasonal and geographical variation).

B. Pressure and Density

The total mass per unit area of the Martian atmosphere has been estimated from the amount of light scattered or the degree of polarization of the reflected light. On the basis of the weighted mean of several modern determinations, de Vaucouleurs has adopted a surface pressure value of 85 ± 4 (p.e.) mb as the most probable value of the surface pressure on Mars. Hess, however, believes that the reliability of this estimate is somewhat less, such that "... it seems reasonable to assert that the surface pressure on Mars probably lies between 70 and 100 mb ..."

The vertical variation of pressure in the lower atmosphere of Mars can be best considered on the basis of an assumed convective equilibrium, wherein an adiabatic vertical lapse rate of temperature would prevail. Then, Poisson's equation relates the vertical change in pressure and temperature as follows:

$$\frac{T}{T_o} = \left(\frac{P}{P_o}\right)^{R/C_p}$$

where:

T = the temperature at pressure, P

T_o = the temperature at the reference surface pressure, P_o

R = the gas constant for the particular atmosphere involved

C_p = the specific heat at constant pressure

The value of R/C_p can be reasonably assumed to be equal to the terrestrial value; the value of T is obtained from the adiabatic lapse rate of temperature, Γ, which is equal to g/C_p, or 3.77°C per km (based upon a Martian gravity value of 377 cm sec^{-2} and a C_p of 0.248 cal gm^{-1} $°C^{-1}$ for pure nitrogen). Because of the lower value of Γ, the Martian pressure decreases

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more slowly than in the Earth's atmosphere, and an equivalence is reached slightly above 25 km (based upon T_o = +10°C and P_o = 85 mb for initial Martian surface parameters), when compared to the NACA standard atmosphere for the Earth.

The vertical distribution of density on Mars can be calculated on the basis of the pressure and temperature values which result from the Poisson relationship. Here again, the cross-over point of density equivalence in the Martian and terrestrial atmospheres occurs slightly above 25 km. It must be cautioned, however, that these calculations are very sensitive to the adopted surface values of T_o and P_o. Nevertheless, the Martian pressure and density probably exceed terrestrial values above an altitude of 20 to 30 km.

C. Temperature

The horizontal distribution of temperature near the base of the Martian atmosphere has been fairly well established as a result of infrared radiometric measurements. As Hess has pointed out, however, these data are subject to several systematic errors if interpreted as the equivalent of standard meteorological temperatures. These errors arise from the assumption of black body emission, from the neglect of absorption and emission of infrared energy by the Martian atmosphere itself, and from the fact that these radiometric temperatures refer essentially to the ground and not to the usual meteorological "surface" temperatures which are measured on Earth at an elevation of approximately two meters. To some extent, these three factors are self-cancelling. Hess has concluded, however, that the radiometric Mars temperatures may be expected to be too high, especially those near mid-day. De Vaucouleurs has commended that the probable temperature error is of the order of $\pm$5°C and that it is unlikely that the absolute errors could much exceed $\pm$20°C.

The most recent radiometric observations reported in the literature are those by Sinton and Strong (1960), who obtained drift curves across the disk of Mars. On the basis of their measurements taken near the equator it was found that the temperatures are near -60°C at 0700 hour, reach a maximum near +22°C at about 1230 hour, and fall during the afternoon. Hess estimates from these data that the nocturnal minimum, occurring near 0600 hour, should be approximately -70°C to -75°C. Thus, in the equatorial region, the diurnal range of temperature approaches 100°C.

Gifford (1956), on the basis of re-evaluated radiometric data obtained from 1926 to 1943, has constructed a graph showing the average noon temperatures on Mars as a function of season and latitude. Table A-2 has been prepared from values interpolated from Gifford's original graph. These values fit fairly well with theoretical expectation. The hazards inherent in this type of analysis are demonstrated, however, by the values shown for 15°, 30°, and 45°S during the Southern Hemisphere spring season, where we have chosen values from Gifford's optional smoothed curve. The original radiometric data, however, indicated a much lower average noontime temperature in the region centered at 16°S, near Hellas, which, it has been suggested, is an elevated plateau and a well-known location of temporary bright markings resembling frost or low cloudiness.

Table A-2. Seasonal Averages of Noon Temperatures on Mars (°A) - After Gifford (1956)

Southern Hemisphere Season	Latitude °S 75	60	45	30	15	0	°N 15	30	45	60	75
Spring	240	247	253*	255*	254*	250	240	230	220	--	--
Summer	243	254	264	272	274	269	261	250	239	226	--
Fall	225	238	251	261	270	274	274	271	265	255	244
Winter	--	---	---	---	275	278	281	283	285	287	---

* Interpolated from Gifford's optional smoothed curve

As de Vaucouleurs has pointed out, night-time surface temperatures can only be roughly estimated on the basis of extrapolated day-time data or theoretical considerations. Since the tenuous, dry atmosphere offers little protection against radiative cooling at night, the night-time minimum temperatures may be expected to reach as low as -70° to -75°C even in the Martian tropics, and rough theoretical estimates suggest average minimum temperatures in winter of the order of -115°C at the north pole and -105°C at the south pole. A lower limit for the minimum temperature on Mars has been calculated as -120°C by de Vaucouleurs on the basis of the assumption that CO_2 does not condense on the Martian surface, even in the polar regions; he does not disallow the possibility, however, that the surface temperatures may attain even lower values during short periods under exceptional circumstances.

The vertical variation of temperature has been reasonably discussed in terms of a model where convective equilibrium prevails in the troposphere and radiative equilibrium in the stratosphere. The height of the tropopause boundary has been variously estimated over a range from 3 to 45 km. In this regard, we accept the Hess arguments which conclude with a height between 30 to 40 km for the base of the stratosphere, where the associated temperature is some 140° to 150°K. Hess criticizes the tropopause height values obtained by Kuiper and others as being too low for two reasons: 1) they adopted too high a value of tropopause temperature, and 2) they incorrectly assumed an adiabatic lapse rate to apply from a base of global mean temperature values, whereas, in reality, the adiabatic lapse rate can only be expected to apply near the equator at mid-day when the surface temperature is at least 40°C warmer than the global mean.

D. Clouds

In visual wavelengths, the Martian atmosphere is usually free of noticeable clouds except near the morning and evening limbs and over the polar caps. From time to time, however, clouds of varying sizes and

colors may be seen over areas far removed from the edges of the planetary disk; these may range from small light spots to extensive clouds covering most of the planetary surface. The cloud observations suggest a division into three main categories: yellow clouds, white clouds, and blue haze (or clouds).

The yellow clouds are commonly agreed to be composed of dust stirred up from the surface by wind storms. Available estimates suggest that they occur at fairly low levels, about 5 km or less, although the tops of some yellow clouds have been occasionally observed at 30 to 35 km above the surface. Yellow clouds seem to occur almost exclusively when the planet is near perihelion, where on several occasions spectacular and extensive dust storms have blotted out surface details over large areas for several weeks at a time. Although the yellow clouds do not exhibit the same color as the orange desert regions from which they presumably originate, this has been explained in terms of the small size of the suspended particles, which would scatter more strongly for short wavelengths than long ones.

The white clouds are usually observed at the sunrise or sunset limbs of the planet, or above the polar regions, particularly during fall and winter, when the polar cap is in the process of being deposted on the surface. The white clouds observed at sunrise frequently do not rotate with the planet, and may thus be low-lying fogs or possibly surface deposits of white frost which dissipate shortly after exposure to sunlight. Dollfus has reported that the polarization curve of these white clouds is identical with that of terrestrial ice crystal clouds. The possible height range for these clouds extends from the surface to some 5 to 10 km.

The blue haze (or cloud) is so called because it is to be found on photographs taken in blue or ultraviolet light and is not detectable in visual wavelengths. Normally the blue haze obscures the Martian surface features in the short wavelengths, but has been observed to clear away on a nearly planet-wide basis within a matter of a few days, remain clear for several days, and then return to normal opacity within another few days. The most

prevalent hypothesis, according to Hess, is that the blue haze is an optically thin, high-level layer of H_2O - ice crystals; Kuiper has shown, for example, how such an H_2O - ice layer, with particle radii of 0.15 to 0.20 microns, could produce the known reflectivity and polarization of the blue haze. As yet, no one has postulated a plausible mechanism which could explain the planet-wide nature of the postulated warming and cooling to dissipate and regenerate these ice-hazes.

E. Atmospheric Circulation

Hess has provided the best meteorological discussion on the Martian wind circulation. As he has noted, the Mars circulation may be simpler than that of the Earth since there are no oceans, presumably less topographical irregularity, and lesser frequency of clouds. Unfortunately, the only way to observe winds on Mars is to follow the drift of clouds. Hess (1949) provided a conjectural analysis of the Martian wind circulation based upon three decades of scattered cloud-drift observations; he found that a set of schematic streamlines could be reasonably fitted to these data, and that the analysis bore a great resemblance to terrestrial flow patterns. De Vaucouleurs has noted that a circulation pattern reminiscent of the trade winds in the southern hemisphere has been observed on several occasions. In general, the observed cloud drift measurements on Mars have provided typical values of 5 mph, with some extreme values of 35 mph or more being recorded in the early phases of a dust storm.

On the basis of the thermal wind equation, which relates the rate of change of wind speed with height and the horizontal variation of temperature across the flow, Hess has made theoretical calculations of the vertical gradient of the westerly component of Martian winds (during the southern hemisphere summer and northern hemisphere winter). The values indicate a vertical increase in wind speed of the order of 2 to 6 mph per kilometer. Thus, Hess concludes "that at elevations of a few kilometers the wind

speeds on Mars are quite comparable to those on Earth at the same height in summer, but are somewhat smaller in winter". This argument is not applicable at higher altitudes.

The longer duration of seasons and the greater orbital eccentricity (affecting the solar radiation received) produce important circulation differences on Mars as compared to Earth. Both factors tend to increase the thermal contrast between the hemispheres in winter and summer, so that a greater seasonal interhemispheric exchange of air must take place on Mars. This is strongly suggested by the observation that each polar cap diminishes to a small size in its summer while the other simultaneously enlarges. Since the storage capacity for water vapor is relatively small in the Martian atmosphere, it would be necessary that the atmosphere transport this moisture from one hemisphere to the other, thus implying a vigorous flow between hemispheres.

F. Ultraviolet Radiation

Although the amount of abiotic ultraviolet radiation reaching the Martian surface is of considerable interest, de Vaucoleurs has noted that we have no direct information on this point. Particle scattering in the blue haze may reduce somewhat the amount of U.V. radiation reaching the surface of Mars, but probably not enough to stop it completely. If so, the surface of Mars may well be exposed to much more abiotic radiation near 2500 Å than on the Earth's surface.

IV. SURFACE PARAMETERS

A. Polar Caps

The polar caps of Mars are not only a very distinct visual feature but their observation by radiometric, spectral and polarimetric techniques has provided a valuable source of information. On the basis of infrared spectra

studies by Kuiper and polarimetric experiments by Dollfus, the polar caps are rather reliably known to comprise a thin layer (perhaps 1 to 5 cm thick, with an order of magnitude uncertainty) of H_2O-frost deposited on the surface. The polar caps are formed during fall and winter by condensation and deposition. At the end of winter the areographic latitude of the edge of the polar cap is about 60 degrees in the southern hemisphere and 70 degrees in the northern hemisphere; the corresponding areas are about 10×10^6 km^2 and 4.5×10^6 km^2, respectively. The more extensive area of the southern cap is caused by the longer and colder autumn and winter in the southern hemisphere (refer to Table A-1). The central "cores" of the polar caps seldom, if ever, vanish completely in summer; the frost deposit on these cores, some 300 to 600 km in diameter, may be 5 to 10 times thicker than over the surrounding region. Of particular interest is the displacement of the south polar cap by some 7 degrees from the areographic pole, as compared to the north polar cap which is practically coincidental.

The seasonal cycle of polar regression does not repeat exactly from year to year, with a given phase of regression being occasionally earlier or later by up to ± 3 weeks. Nevertheless, the order of topographical events in the retreat of a polar cap repeats with great regularity. In particular, bright regions that appear near the edge of the receding cap in spring probably indicate the presence of local relief favoring cloud condensation and retarding the evaporation of the surface deposit.

As pointed out by de Vaucoleurs, at any moment more than 90 percent of the total mass of water on Mars (i.e., 10^{16} to 10^{18} grams) is locked up in the polar caps and only a small fraction is dispersed in the atmosphere. For all practical purposes, there is no free water vapor in the Martian atmosphere, thus effectively excluding the possibility of hydrometeors. As de Vaucouleurs notes, "Clouds and polar mists must be more comparable to a very dry haze of submicroscopic ice crystals and the polar surface deposit to a powdery precipitate of very minute grains".

B. Bright Regions

Some three-quarters of the Martian surface consists of bright reddish or orange areas, most generally referred to as deserts (or "continents") and considered to be covered with a very dry fine dust. Kuiper, on the basis of infrared spectrophotometry, has proposed that this desert dust is felsite, a fairly common, fine-grained brownish igneous rock. On the other hand, Dollfus has studied the visual polarization curve of Mars and found a terrestrial counterpart in pulverized limonite, a common hydrated iron oxide, which has the same color and (visual) albedo as the bright regions of Mars. As stated by de Vaucouleurs, no definite choice is yet possible between the conflicting reports. Hess mentions that this situation is not surprising, since neither reflection spectra nor polarization curves for solid surfaces provide the kind of unique identification supplied by transmission spectra of gases.

The Martian desert areas are commonly taken to be the source of suspended fine particles which comprise the yellow clouds. In this regard, the recent analysis by Barbashov and Garazha (1960) of the brightness distribution curves during the 1956 opposition is most interesting. Among their conclusions are the following: 1) The surface of Mars is overlaid by extremely minute dust-like particles whose grain diameters do not exceed 10 to 100 microns, 2) the dust clouds on Mars apparently consist of still finer particles, 3) dust particles rarely ascend to the high atmospheric layers, 4) the yellow haze (or cloud) apparently consists of particles of the same grain size, or finer, as the particles covering the Martian continents (bright desert areas).

C. Dark Regions

The dark areas of Mars form a conspicuous and fairly stable platform of maria (seas), canals and oases distributed over the planetary surface. Although there has been substantial agreement on those more prominent

features which have been mapped in great detail, there are random changes in outline and intensity, as well as seasonal changes in coloring, which have provided a source for very imaginative speculation.

The most substantial piece of evidence regarding the makeup of the dark areas has been Sinton's discovery (1959) of characteristic C-H vibration absorptions near 3.5 microns in the reflection spectrum of the dark maria (these absorptions were found to be absent in the spectrum of the orange deserts). Thus, the presence of organic matter on Mars is quite probable, even though no evidence has been found for the characteristic infrared reflection spectrum of chlorophyll in the dark areas (chlorophyll may still be present, however, since certain terrestrial plants have natural coatings which prevent the detection of this reflection spectrum).

Another important characteristic of the dark regions is their seasonal variations in albedo, polarization, and possibly color, all related to the growth and decay cycle of the polar caps. These seasonal variations are usually regarded as associated with the atmospheric transfer of water vapor from pole to pole and have been frequently interpreted as evidence for the existence of Martian plant life.

Another revealing property of the dark regions is the occurrence of irregular non-seasonal variations. As de Vaucouleurs has commented, the reality of such changes, attested by reliable visual and photographic observations, is beyond doubt. In 1954, for example, Slipher and others photographically recorded a new dark area covering almost 600,000 square miles. Occasional short-lived but strong dark spots have been seen to appear (and photographed) in bright areas.

In recent years, the nature of the dark regions has been generally discussed in terms of the vegetative, or the volcanic hypothesis. The vegetative hypothesis is supported by: 1) the seasonal variations in the dark areas, 2) their apparent dependence on water vapor, 3) the polarizing properties, 4) the presence of an infrared absorption band characteristic of

organic molecules, 5) the persistence of the dark regions in spite of aeolian deposition of desert dust, and 6) evidence that certain terrestrial microorganisms can survive in a simulated Martian environment.

Kuiper (1951) summarized the evidence to support the vegetative hypothesis as follows: 1) the colors are not those displayed by inorganic substances in surroundings of about -30°C and low humidities, 2) the colors are not all alike but show regional patterns similar to those found on Earth, 3) the colors change seasonally, apparently as a result of seasonal changes in moisture distribution, and 4) Opik's argument is convincing that the green areas would have long since been buried by the yellow dust if they lacked regenerative power.

The volcanic hypothesis advanced by McLaughlin (1954) has not received as much popular support. It assumes that active volcanoes are sources of vast amounts of ash and cinders which are carried by the prevailing winds and are deposited to form the dark areas. In support of this hypothesis are cited the non-seasonal variations which can be resolved by the wind-deposition hypothesis, if temporary volcanic activity is assumed in appropriate places. There are important objections to this theory as a sole means for explaining the changes in the dark areas, but it may constitute a secondary but important means for modifying the primary distribution of regenerative vegetation.

D. Topography

The degree of topographical vibration to be encountered on the Martian surface is somewhat uncertain. Presumably, any high mountains could be detected as projections or detached bright spots along the terminator (border between night and day). Thus, such isolated features probably do not exceed a few thousand feed in elevation. On the other hand, the regularly repeated anomalies in the shape of the south polar cap during its regression and certain preferred locations for abnormal brightening suggest the

existence of definite topographical features. Even though isolated peaks have not been detected along the terminator, gradual scopes may extend over wide areas and produce departures from the spheroid of several thousands of feet.

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I. IDENTIFIABLE CHARACTERISTICS OF TERRESTRIAL MICRO-ORGANISMS

On Earth, the diverse morphology and variable physiological capacity of biological specimens are well recognized. Nevertheless, all living organisms that now exist (with the exception of viruses) exhibit a number of common basic characteristics:

1) A common chemical composition

2) A common physical organization into cells

3) Common chemical activities

4) Capacity for growth

5) Capacity to adjust to environmental changes

A. Chemical Composition

Element	Percent in Living Matter
O	76
C	10.5
H	10.0
N	2.5
P	0.3
K	0.3
S	0.2
Mg	0.02
Ca	0.02
Fe	0.01

APPENDIX B

INFERRED PROPERTIES OF MARTIAN MICROORGANISMS

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Class of Substance	Percent by Weight	Approximate Molecular Weight	Relative Molecular Abundance
Water	83	18	4,500,000
Protein	10	100,000	100
Nucleic Acids	1	1,000,000	1
Lipids	2	500	4,000
Carbohydrates	2	500	4,000
Other Organic Substances	0.1	250	400
Minerals	1.9	100	19,000

Analytical techniques which are sensitive enough to detect protein, nucleic acids, lipids, and carbohydrates can, therefore, be used to infer the presence of living material.

B. Physical Organization

Most living organisms are composed of microscopic units (cells) with a definite size and shape, each bounded by one or more membranes. The tremendous diversification of structure in different living organisms is a result of two things: variation in structure of individual cells and variation in arrangement of cells into organized groups. Organisms can consist of single cells (e.g., bacteria, protozoa, algae) or in multicellular structures ranging from 10^2 cells/individual (rotifers) through 10^3 (hydra) to 10^{13} (man). Microbial cells range in size from 0.25μ x 0.4μ (Rickettsia) to 5 mm (Pelomyxia carolinensis). Most microbes normally found in the terrestrial environment as free living autotrophes and saprophytes will be in the size range of 1 micron to 100 microns.

C. Chemical Activities

Living creatures have the ability to use energy yielding chemical reactions to produce order (organization) out of chaos. All free living microbes have the capacity to produce detectable chemical and physicochemical changes in their environment.

D. Capacity for Growth

All viable microorganisms, at one stage or another in their existence, demonstrate the ability to grow and reproduce. These phenomena are manifested by a simple increase in cell size and volume, an actual fission to yield two or more daughter cells from one parent, the production of specialized reproductive spores which can germinate, the pinching off from parent cells of fragments which will themselves grow and reproduce, or by a sexual process in which two cells will mate to yield progeny. In each case, growth and reproduction is demonstrated by an increase in cellular protoplasm.

E. Capacity to Adjust

All viable microorganisms or their progeny will demonstrate the ability to adapt to environmental changes and stresses. This ability to adapt, however, is only tenable within certain limits of the physical and chemical environment. Ultimately all living cells will die, their physical organization will be disrupted, their complex biochemical structure will be degraded to simple oxidized elements, and their chemical and growth potential will be lost.

II. IMPLICATIONS FOR EXOBIOLOGY

The demonstration, on Mars, of entities which exhibit all five of these characteristics will be obvious proof of the presence of living organisms. This demonstration, however, will depend on more sophisticated techniques than are feasible with a microscope system. Nevertheless, a Martian microscope can be employed to evaluate the following criteria:

1) Spectrophotometric analysis of chemical composition.

2) Presence of physically organized cells, and cell masses with varying degrees of organizational complexity (i.e., nucleus, structures) and function (i.e., motility).

3) Chemical changes which are optically demonstrable such as peptonization of protein, degeneration of polysaccharides, and pH and Eh changes in the presence of sensitive dyes.

4) Increase in cell numbers or mass upon culturing.

5) Change in size and shape of cells and progeny under environmental stress.

The easiest characteristic to study, using the simplest automatic manipulations and optical system with the greatest probability of success, will be No. 2 – the detection of organized particles in the size range of 1 to 5 microns. If one finds artifacts analogous to terrestrial cells, and clumps of these artifacts, a major criterion for the presence of Martian life will be met. More sophisticated chemical, biological and spectrophotometric techniques can be used to supplement and enforce the original observation. Conversely, it will be difficult to satisfy the stated requirements for presence of living material if cells and cell fragments are not demonstrated. Even if analysis demonstrates the presence of biochemicals, a dynamic change in the physical chemistry of the environment and increase in turbidity or opacity of a liquid, the existence of life will remain debatable unless a physical organization of some sort can be detected.

III. SPECULATION ABOUT MARTIAN BIOLOGY

Salisbury (Science 136: 17, 1962) reviewed the positive and negative evidence for presence of biological entities on Mars and listed certain criteria which would have to be met by living organisms to account for astrophysical phenomena about which there is general agreement. On the basis of his analytical reasoning, he constructed a theoretical model of plant life which met those criteria. Essentially he postulated a flat leaf-like structure of uncertain size which would be sufficiently tensile to roll up and uncurl and which would probably be a fraction of a millimeter thick.

This creature, or fragments of such creatures would not be unlike certain terrestrial fungi in physical organization – though certainly different physiologically. If terrestrial fungi can serve as physical models (Basa and Hawrylewicz, ARF 3194-4, 1962) one might then project the thought that atmospheric and surface dust samples would be probable areas for their detection. Furthermore, until more is known, one must assume that we will be dealing with cells and cell masses whose size and relative appearance will be of the same order as terrestrial fungi.

IV. MICROBIAL CONCENTRATIONS IN SOIL AND AIR

The bacterial content of a sample of soil depends upon a variety of interacting factors, such as moisture content, depth of sample, aeration, organic matter content, etc. Furthermore, the method of enumeration (culture or direct count), medium and incubation conditions all will influence the reported values. It is generally agreed, however, that a count range of 10^5 - 10^8/gm of soil, and an average count of 10^6 - 10^7/gm can serve as useful rules of thumb in describing microbial contamination of soil.

Much less information is available for airborne microbes. Since, to our knowledge, microorganisms do not proliferate in the airborne state but are rather incidental contaminants, their concentration in air depends

largely on meteorological considerations, the degree of contamination in soils, the ease with which surface contamination may be made airborne, and the factors which determine the physical and biological decay of viable airborne particles. On the basis of our studies with volumetric air samplers, it would be fair to quote counts of 1 to 50/cubic feet of air for the extramural environment through the year, with a reliable mean of 10 to 20/ cubic feet. (These are minimal figures since they refer to contaminated particles. The actual total number of individual organisms that could be microscopically observable would be higher.)

In order to convert the airborne count (volumetric) and the soil count (gravimetric) to an equal basis, it is necessary to know the relative concentration of total solids in each system. General figures for soil composition show a mineral content of 45 percent, water 30 percent, air 20 percent, and organic matter 5 percent. Particle size distribution in soils range as follows:

Diameter Limits	Sandy Loam	Heavy Clay
2 - 0.2 mm	66.6%	0.9%
0.2 - 0.02 mm	17.8%	7.1%
0.02 - 0.002 mm	5.6%	21.4%
< 0.002 mm	8.5%	65.8%

In air, a value of 0.03 grains of dust/1000 cubic feet is a representative figure. Thus the microbial content per gram in each system can be compared as follows:

SOIL: $10^6 - 10^7$ viable organism/gm of solids

AIR: 10 - 20 organisms/cu ft

0.03 grains dust/1000 cu ft = 2×10^{-6} gm dust/ft^3

2×10^1 organisms/2×10^{-6} gms dust

1 organism/10^{-7} gm = 10^7 viable organism/gm solids

When calculating the ratio of viable organism per particle of inanimate matter, several assumptions need to be made:

1) Microbial distribution is uniform and random.

2) Microbes are associated with inanimate particles > 1-micron diameter.

3) Dust and soil are dry and dispersible.

For air: Concentration of total particles $> 1\mu = 10^5/ft^3$

Concentration of viable organism = $10^1/ft^3$

organism/particle $\approx 10^{-4}$

For soil: 1 gm with Sp Gr 2.65 = 0.377 cm^3

assume 20% air, solid matter occupies 0.301 cm^3 per gm

assume mean particle diameter of 2μ, 1 gm contains 1.5×10^{10} particles

assume 10^6 viable organism/gm, organism/particle $\approx 10^{-4}$

Therefore, viable contamination level per gm of solid matter and per inanimate particle is similar in both soil and air.

APPENDIX C

SAMPLE COLLECTION

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I. INTRODUCTION

Our studies have indicated that the adoption of the terrestrial analogy regarding probable Martian microorganisms is the best that can be made at this time; thus, the particles which we are most interested in sampling fall within the general size range of 0.5 to 50 microns and density range of 1.0 to 1.2. The next fundamental question to be resolved is from what portion of the Martian environment should samples be obtained to provide the best chance for detecting elementary forms of life: from the atmosphere, from the surface, or from below the surface? In this connection, our studies have led us to the reasonable assumptions that 1) the viable biosphere, if any, is probably dependent upon solar radiation as the primary energy source, with but limited energy transfer processes being involved, and 2) microorganisms and primitive forms of plant life will probably thrive best in those areas where the H_2O constituent of living matter can be placed into a liquid phase for the longest possible periods on a regular daily basis.

If we further consider the fact that the mean daily temperature, even in the equatorial regions, is considerably below the freezing point of water, and if we discount the possibility of significant sources of interior heat being transported toward the Martian surface, then it would appear doubtful that viable microorganisms can be concentrated in any appreciable quantity anywhere else than at the atmosphere-ground interface. It would thus seem that drill samples from any appreciable depth are probably not justified on an initial life-detection probe and that a simpler means for atmospheric and selective surface sampling of naturally occurring small particles represents a more feasible approach. A further argument can be made for the latter type of sampling approach in terms of the characteristics of the Martian wind circulation which appear favorable for the airborne dispersal of small particles in the size range of interest. Thus, the Martian atmosphere conceivably acts as a means for the long-range transport of living

organisms from preferential source regions, so that sampling of particles from the air itself or from the eventual surface deposition of aerosols probably affords the method which will be most generally successful, no matter where the probe should land.

II. BASIC APPROACHES TO SAMPLE COLLECTION

As we have chosen to define it, the term sample collection includes the following aspects: 1) initial acquisition of particulate matter, 2) transport of the acquired material to a collecting medium, and 3) deposition of the particulate matter onto a collection surface or into a collection container.

Although the problem of sample collection can be approached in various ways, the two basically different methods are 1) mechanical and 2) pneumatic. With the mechanical method, the material would be dislodged from the surface by drilling, scraping, grinding or digging; the material would be mechanically enclosed or confined; and finally, the sample would be mechanically transported to a deposition point where the material would be released into a hopper.

In the case of the pneumatic method, particulate matter which has been aerosolized, either naturally by the Martian wind or artificially by a sweeper-brush or jet of air, is transported by the gas in which it is suspended. The transport is effected by pulling or sucking the gas through an enclosure such as a simple duct or tube. At the desired deposition point, an inertial or electrical force is applied which causes the gas-borne particle to move out of the gas stream and onto a collection surface. A system which would use a liquid rather than a gas for particle transport is also possible but is not considered practical for our application.

We have adopted the pneumatic approach to sample collection since it provides the most generally applicable, simplest and inherently most reliable method to obtain specimens for microscopic observation and detection.

In comparison to mechanical methods, it is realized that pneumatic sampling cannot rapidly provide larger samples of a gram or more, such as required for the multivator and similar bulk analyzing instruments. On the other hand, pneumatic collection of natural or latent aerosols will provide a highly selective sample of particles which are already within the optimal size range for microscopic analysis (thus obviating further treatment in terms of size reduction). Furthermore, the sample collection capability by the pneumatic method is of the order of a milligram for natural aerosols and considerably more for latent aerosols; and a milligram sample, if properly refined with no significant losses of microbiological constituents, is considered to be quite adequate for providing a reasonably good specimen if suitably concentrated within the field-of-view of the microscope. In addition, the aerosol sampling concept is less limited by the local, specific nature of the Martian surface which may be present in the vicinity of the instrument landing site.

Although a quantitative trade-off comparison of the possible sampling methods would be desirable, an extensive analysis of the relative power requirements and collection efficiencies for competitive mechanical methods is beyond the scope of this study. Preliminary analysis has shown, however, that the large variety of possible substrates (extending from hard rock to fine sand) will produce a corresponding large range in the estimated power requirements, if the implications of size reduction techniques are properly considered, and if realistic terrain factors are incorporated into the model.

It should be pointed out, moreover, that our adoption of the pneumatic sampling method has in no way imposed limitations upon the type of processing and observational techniques which can subsequently be used. It is conceivable, for example, that a more extensive ground-sampling method may be used to collect very large amounts of surface and sub-surface material and to feed hopper samples to several instrument probes. In this event, a hopper sample of natural or reduced particles would be compatible with the general design concept of our processing unit.

III. BASIC PROPERTIES OF AEROSOLS

There are some basic properties of aerosols which will now be briefly reviewed. If we first consider the settling velocities for particles exposed to a Martian density and gravity environment, the results are as shown in Figure C-1. We may note that the settling velocities for Martian particles in the size range of 1 to 100-micron diameter are lower by an average factor of 2, as compared to terrestrial aerosols. Thus, on a theoretical basis the lower Martian atmosphere is a favorable medium for the suspension and transport of aerosols. Other theoretical aspects of Martian meteorology and observational evidence of yellow cloud phenomena lend support to the idea that small, loose, dry surface particles are being continuously aerosolized, transported, and deposited on a planet-wide basis; furthermore, the lack of significant precipitation processes on Mars prevents scavenging and washout of airborne particles.

In Figure C-2 is shown a chart which relates the mass of collected aerosol sample to the sampling rate, sampling time, and mass concentration. If, as an example, we consider a 9.5 cfm aerosol collection unit, it may be seen that approximately a 30-minute period would be required to sample 300 ft^3 of ambient air, which would contain on the order of 1 milligram of aerosol particles (assuming a mass concentration of 3.3 $\mu g/ft^3$, a value of aerosol concentration typically observed during moderately dusty conditions near the Earth's surface). A milligram sample per operational cycle can be taken as a reasonable order-of-magnitude estimate for the capability of a Martian aerosol collection system.

Another basic aspect of aerosol collection and analysis is the particle size distribution. The work of Whitby et al.* provides an authoritative estimate of typical airborne particle distribution in terms of percent by weight,

* Whitby, K. T., et al. The ASHAE air-borne dust survey. Heating, Piping and Air Conditioning, ASHAE Journal Section. p. 185-192 (Nov. 1957).

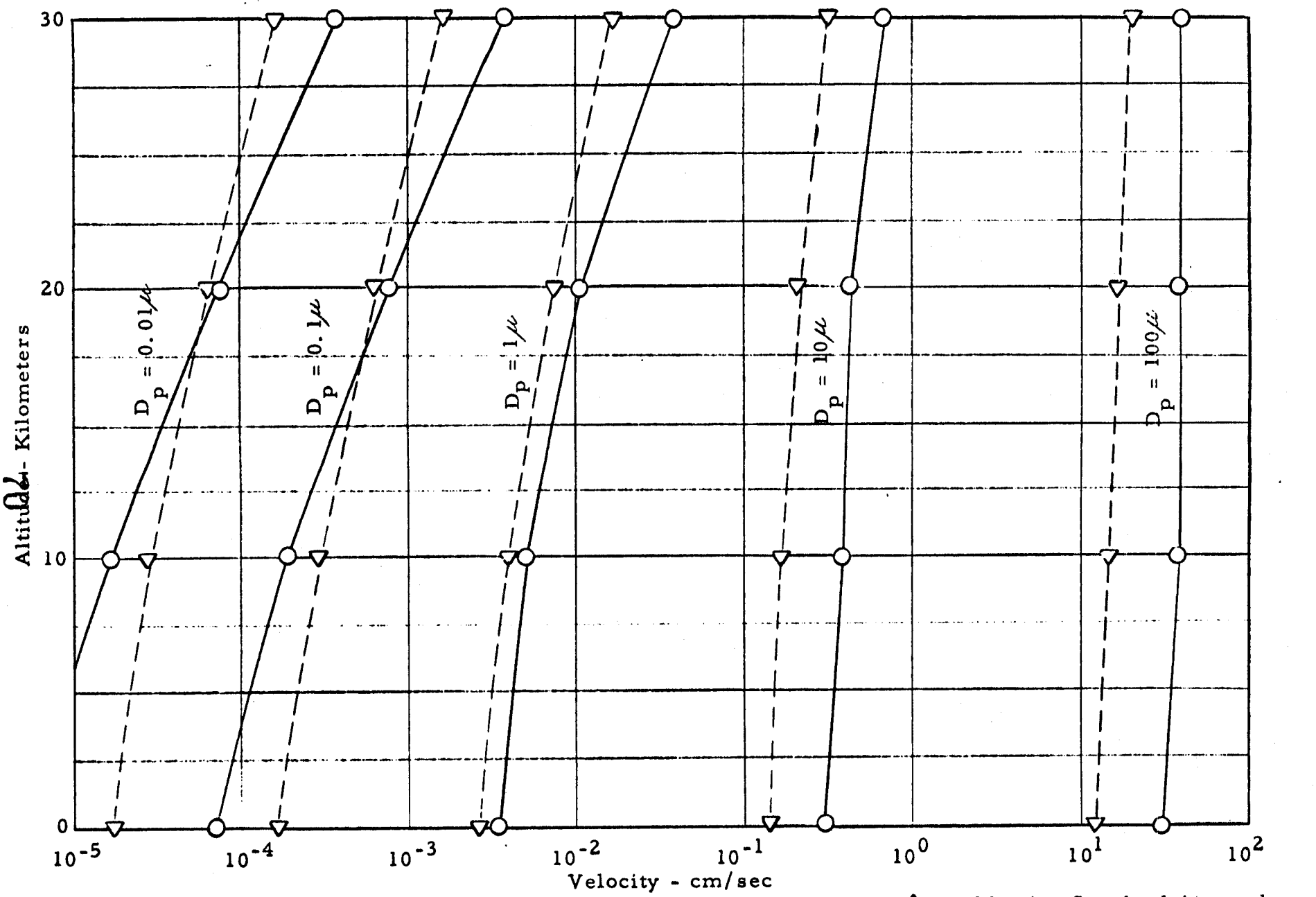

Figure C-1. Terminal Gravitational Settling Velocity for Spherical Particles of Density 1.0 (calculated from Stokes-Cunningham Law)

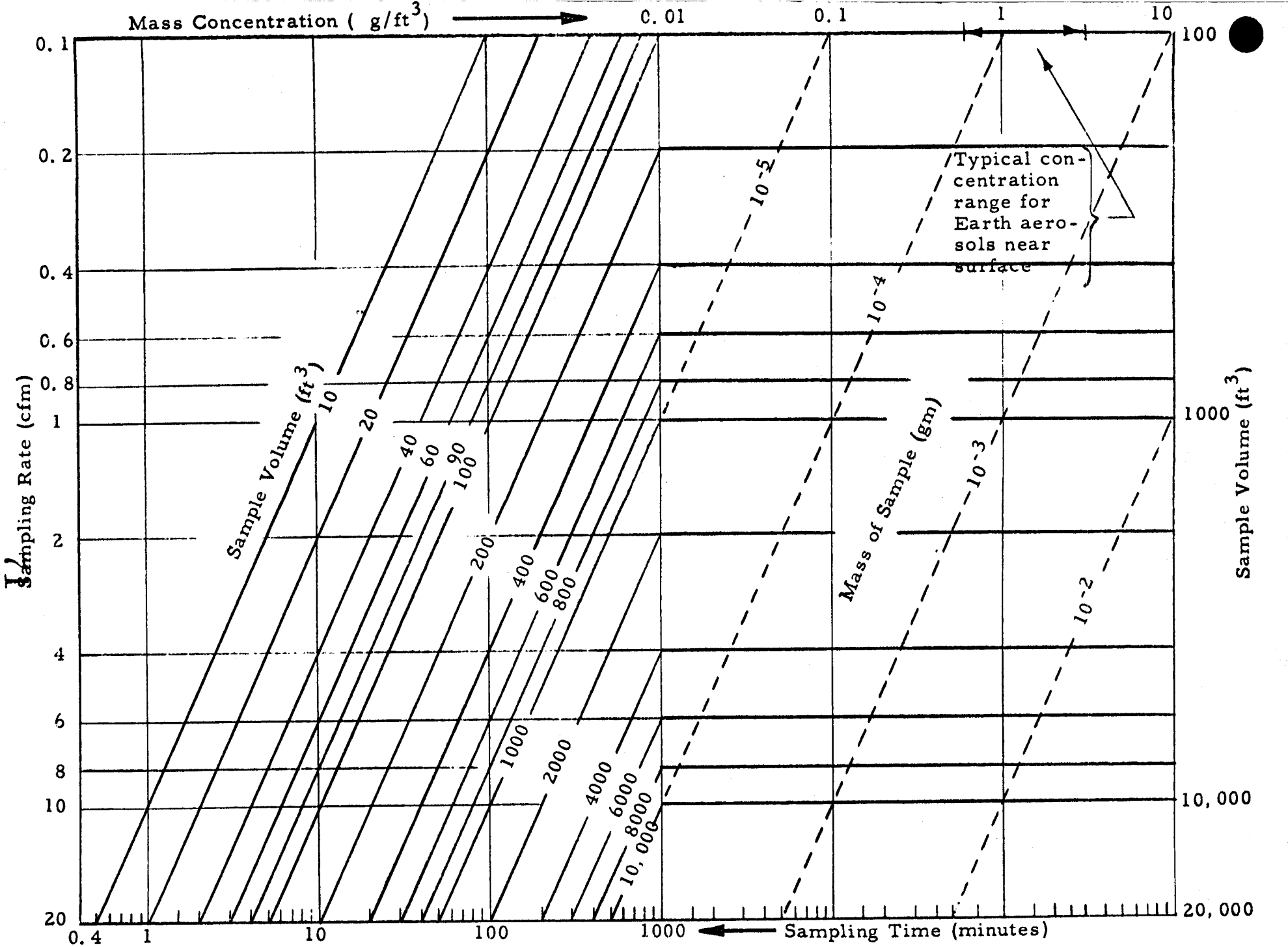

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Figure C-2. Chart Relating Mass of Aerosol Sample with Sampling Rate, Sampling Time and Mass Concentration

area, and number; interpolated values for particle sizes of interest to this study are shown in the following table:

Particle Diameter (μ)	Percent of Aerosol Sample		
	By Weight	By Area	By Number
< 0.5	10	48	98.1
< 1.0	22	70	99.6
1.0 - 2.0	15	14	0.35
2.0 - 5.0	30	11	0.049
> 5.0	33	5	0.001

Thus, if we assume that this terrestrial data crudely approximates the properties of Martian aerosols, then, with a minimum size cutoff of 0.5 micron, some 90 percent by weight of the potential aerosol sample would be collected, even though it might contain only 2 percent by number of the particles.

Another important problem area affecting the design of an aerosol collector is the extent to which small microbes "hitch-hike" on larger mineral particles. If microorganisms were to occur predominantly as discrete individual specimens, it would be expeditious to use multiple impactors to perform an initial size separation during the collection process. We might, for example, collect aerosols directly in three different size ranges and observe each size sample (after density fractionation) under high, medium, and low magnification. Unfortunately, definitive studies have never been made under extra-mural conditions to measure the size distribution of mineral particles to which particular species of microbes will adhere. A rough estimate is possible, however, on the basis of a recent study* of hospital air; some representative results of this study are presented in Table C-1.

*Greene, Vesley, Bond, and Michaelsen. Microbiology of hospital air. II. qualitative studies. J. Bact. in press (1962).

Table C-1. Relative Frequency of Microorganisms Collected in Association with Different Sizes of Aerosol Particles

Microorganism	Aerosol Particle Diameter (μ) 1-2	2-6	>6
Micrococci (1μ)	27	30	43
Gram-negative rods ($1 \times 2\mu$)	26	28	46
Actinomycetes (1μ)	68	10	22
Molds (3μ)	5	61	34

These results suggest that clear-cut initial size separation of the microbes themselves by multiple impaction is probably not feasible. Presumably, it is only after the microbes have been dissociated from their micromatrix that their size separation can be carried out effectively.

IV. AEROSOL PARTICLE COLLECTION SYSTEMS

Sample acquisition from the atmosphere requires a means for collecting particulate material from air which has been directed into a suitable sampling device. The operation of continuous aerosol sampling devices depends upon provisions for inducing a flow of air through the system as well as a means for collecting or removing particulate material from the airstream. For example, filter or impactor sampling systems fall into this category. Although sampling devices are occasionally used which do not require an air mover (e.g., sedimentation samplers) these devices are quite limited in volumetric capacity.

For sample collection on Mars, the sampling system must be reliable, small in size and of minimum weight and must be capable of sampling a large volume of air for a given input energy. Since it has been found that considerable processing of the sample is likely to be required prior to microscopic examination, the collection system must also be compatible with processing requirements.

A number of techniques are available for collecting particulate material from an aerosol, e.g., filtration, inertial impaction, sedimentation, thermal or electrostatic precipitation, etc. The most promising of these particle collectors are discussed briefly below.

A. Inertial Impactor

A typical impactor configuration is shown in Figure C-3. The aerosol is accelerated in the nozzle to a velocity V_n and directed against an impaction surface which collects particles having sufficient momentum to carry them to the surface. Both theory and experiment show that the collection efficiency of an impactor of given design depends on a single parameter, the "inertia parameter" ψ, defined by the expression:

$$\psi = \frac{1}{18} \frac{\rho_p C V_n d_p^2}{\mu L} \qquad \text{(C-1)}$$

where:

d_p = the particle diameter

ρ_p = the particle density

μ = the gas viscosity

C = the molecular slip parameter

L = a characteristic dimension of the impactor (e.g., the nozzle diameter for a circular impactor)

The collection efficiency of the impactor is defined as the ratio of the number of particles retained on the impaction surface to the number entering the impactor. A typical plot of collection efficiency versus the inertia parameter ψ is shown in Figure C-3.

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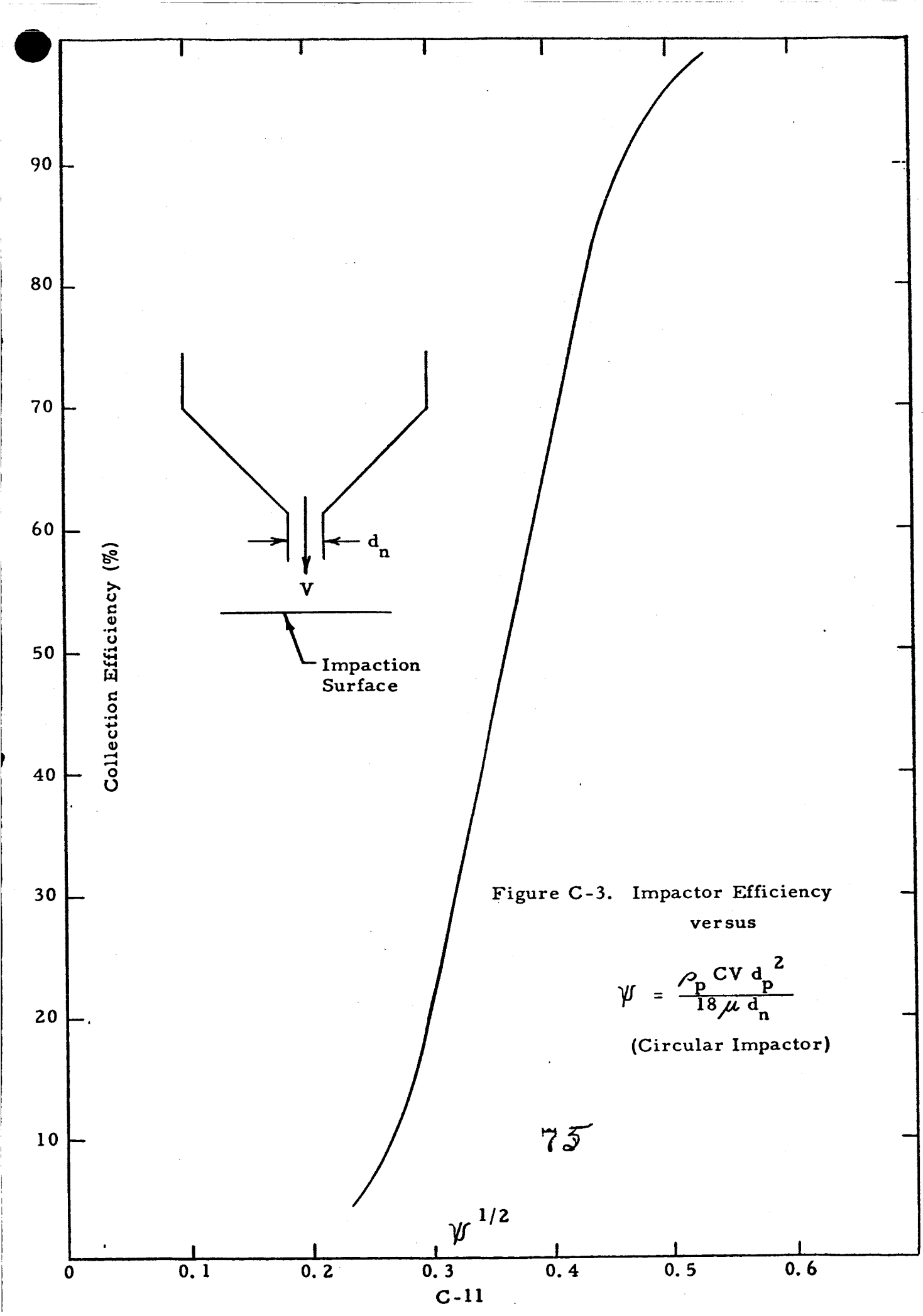

Figure C-3. Impactor Efficiency versus

$$\psi = \frac{\rho_p C V d_p^2}{18 \mu d_n}$$

(Circular Impactor)

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By way of illustration, suppose we wish to design a circular impactor for sampling the Martian atmosphere. Since we are primarily interested in collecting particles with $d_p \geq 0.5$ micron and densities $\rho_p \geq 1.0$, we may stipulate for design purposes that the impactor have a "cutoff" (i.e., 50 percent collection efficiency) for particles with $d_p = 0.5$ micron and $\rho_p = 1.0$. Taking the atmospheric density as about 1/10 that of the Earth's surface, the slip parameter $C \approx 6.7$. Assuming also that $\mu \approx 1.6 \times 10^{-4}$, Equation (C-1) yields:

$$\frac{V_n}{L} = 2.2 \times 10^4 \text{ sec}^{-1} \qquad \text{(C-2)}$$

If the nozzle velocity V_n and nozzle diameter L satisfy this equation, the collection efficiency will be 95 percent or better for all particles with $d_p \geq 1.0$ micron and $\rho_p \geq 1.0$.

The impactor system requires an air mover in order to produce the required nozzle velocity. An analysis of a centrifugal blower for an impactor system is discussed in Section V of this appendix. This analysis shows that the desired impactor cutoff characteristics are compatible with a centrifugal blower air mover. The sampling rate, the required pressure rise across the blower and the nozzle diameter of the impactor are found to depend on the power input to the blower motor through the relationship:

$$Q = 2.7 (\eta_e w)^{0.6} \qquad \text{(cfm)}$$

$$\left(\frac{\Delta p}{p_a}\right)_b = 0.085 (\eta_e w)^{0.4} \qquad \text{(C-3)}$$

$$L_n = 0.465 (\eta_e w)^{0.2} \qquad \text{(cm)}$$

In these expressions, η_e is the overall efficiency of the motor and blower and w is the power supplied to the motor in watts. The ratio $(\Delta p/p_a)_b$ is the pressure increment across the blower divided by the ambient pressure.

For an input power of 20 watts and an assumed efficiency of 10 percent, we find:

$$Q = 4.1 \text{ cfm } @ \left(\frac{\Delta p}{p_a}\right)_b = 0.11 \ .$$

The nozzle diameter is 0.48 cm. If it is assumed that a cutoff of 2.0 microns is permissible, the sampling rate is increased to $Q \approx 9.5$ cfm, while the nozzle diameter becomes $d_p \approx 1$ cm (assuming the same power input and efficiency).

In summary, it appears that an inertial impactor has a number of commendable features as a sample collector for the Mars Microscope system. These advantages include:

1) The impactor will not collect unwanted particles smaller than a certain cutoff size fixed by the impactor design.

2) The collected particulate material is deposited on a relatively small surface area where it can either by viewed directly or subjected to processing (e.g., density separation, concentration).

3) An impactor sampling system can be small and light in weight.

4) Reliability of the impactor system would be very good since it depends only on the air mover for proper operation.

5) An impactor system is capable of sampling at a high volumetric rate with practicable input power levels.

B. Electrostatic Precipitator

The methodology of electrical collection has also been applied to airborne particulate matter. For example, if a system were to involve the collection of airborne particulate matter with the stipulation of a subsequent density separation, it would be convenient and expedient to collect the particulate matter directly onto a liquid surface or onto a gelatin-like surface which would be later dissolved. A sketch of such a collector is shown in Figure C-4.

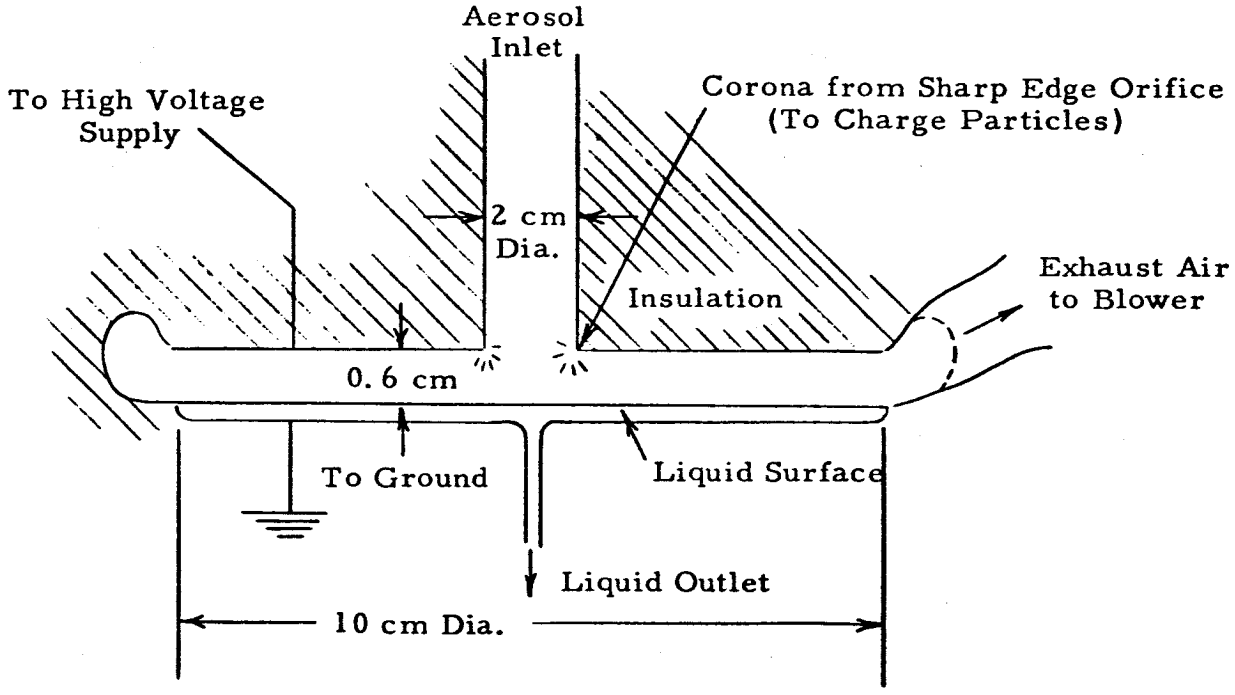


Figure C-4. Schematic of Electrostatic Precipitator

Assume particles of one-micron diameter and larger are to be collected with essentially 100 percent efficiency. The electric charge that can be imparted to a particle is approximately proportional to the particle surface area, or a function of the particle diameter squared for a spherical particle. The fluid viscous drag force acting on a particle is approximately proportional to the first power of diameter. For this collection case only electrical and drag force need be considered; other forces will be second order. Equating the electrical force to the viscous drag force gives:

$$Ee = \frac{3\pi\mu D_p v}{C} \qquad \text{(C-4a)}$$

where:

E = electric field (stat volts/cm)

e = particle charge (stat coulombs) (2.1×10^9 electrons = 1 stat coulomb)

μ = fluid viscosity (poise)

D_p = particle diameter (cm)

v = particle velocity (cm/sec)

C = Cunningham slip correction factor (dimensionless)

Solve for v to get:

$$v = \frac{Ee\,C}{3\pi\mu D_p} \qquad \text{(C-4b)}$$

For a given collector configuration the field strength, E, is fixed but the particle charge, e, varies as the particle surface or as D_p^2. Therefore, v increases as D_p increases.

A calculation for the electrical collection of a one-micron diameter gas borne particle represents the most difficult collection case of interest. The maximum equilibrium charge for a one-micron particle is about 2000 electrons (Langer, G. and J. H. Radnik, J. Appl. Phys. 32, 5: 955-57, 1961). A charge of 500 electrons per particle (a charge which can be obtainable with a properly designed particle charger section) will be assumed for the calculation. A voltage gradient of 3000 volts/cm = 10 stat volts/cm is taken as a reasonable high voltage gradient; flash-over potential at a gas density of 1×10^{-4} g/cm^3 is about 5000 volts/cm. For nitrogen gas at a temperature 15°C, the viscosity is 1.8×10^{-4} poise. The Cunningham correction factor is 2.1 for a one-micron particle on Mars. Therefore, the drift velocity:

$$v = \frac{Ee\,C}{3\pi\mu D_p} = \frac{(10)(500)(4.8 \times 10^{-10})(2.1)}{3\pi(1.8 \times 10^{-4})(1 \times 10^{-4})} = 30 \text{ cm/sec}$$

If a 0.6 cm spacing is assumed for the collector section, then the time for collection will be $0.6/30 = 2 \times 10^{-2}$ sec. If all particles are to be collected from 2500 cm^3/sec of gas (about 5 cfm) then the collection volume must be $(2500)(2 \times 10^{-2}) = 50$ cm^3. For a collector of the type shown, a disk of diameter D_d is required where:

$$(0.6)\left(\frac{\pi}{4}\right)(D_d)^2 = 50 \text{ cm}^3 .$$

or

$$D_d = \left(\frac{(4)(50)}{(\pi)(0.6)}\right)^{1/2} \cong 10 \text{ cm}$$

A collector of this type would require a centrifugal blower capable of moving 5 cfm across a very low pressure difference. The power requirement for a blower of this type should be about 2 watts. In addition, a high voltage supply capable of delivering about 100 microamps output at 2000 volts is required. The high voltage supply power requirement would be less than 1 watt.

C. Filtration

An alternative possibility for obtaining a sample of particulate material from the atmosphere is to use a filter in conjunction with a suitable air mover. Filters used for removing particles from gas streams are usually of high porosity, i.e., the volume of fibers within the filter is a small fraction of the filter volume, on the order of 10 percent for many filters. This type of filter is essentially a compromise between high particle collection efficiency and minimization of power requirements and size of the filter system.

Particles are removed from the gas through the operation of several mechanisms, the most important of which are: inertial impaction, sieving, diffusion (Brownian motion) and electrostatic deposition. Sieving is responsible for removing particles too large to pass through the spaces between individual fibers of the filter. Collection occurs by inertial impaction when small particles which otherwise would penetrate the filter are carried by their momentum to the fiber surfaces where they are held by surface forces. The effectiveness of inertial impaction increases with increased flow velocity. On the other hand, diffusion and electrostatic collection mechanisms are effective only at low flow speeds. Particle collection by impaction, diffusion and electrostatic mechanisms are improved at low gas densities because of molecular slip effects.

A typical filter collection efficiency curve is shown in Figure C-5, illustrating the effects of filter face velocity and particle size. The filter efficiency at intermediate speeds can be raised by increasing the thickness

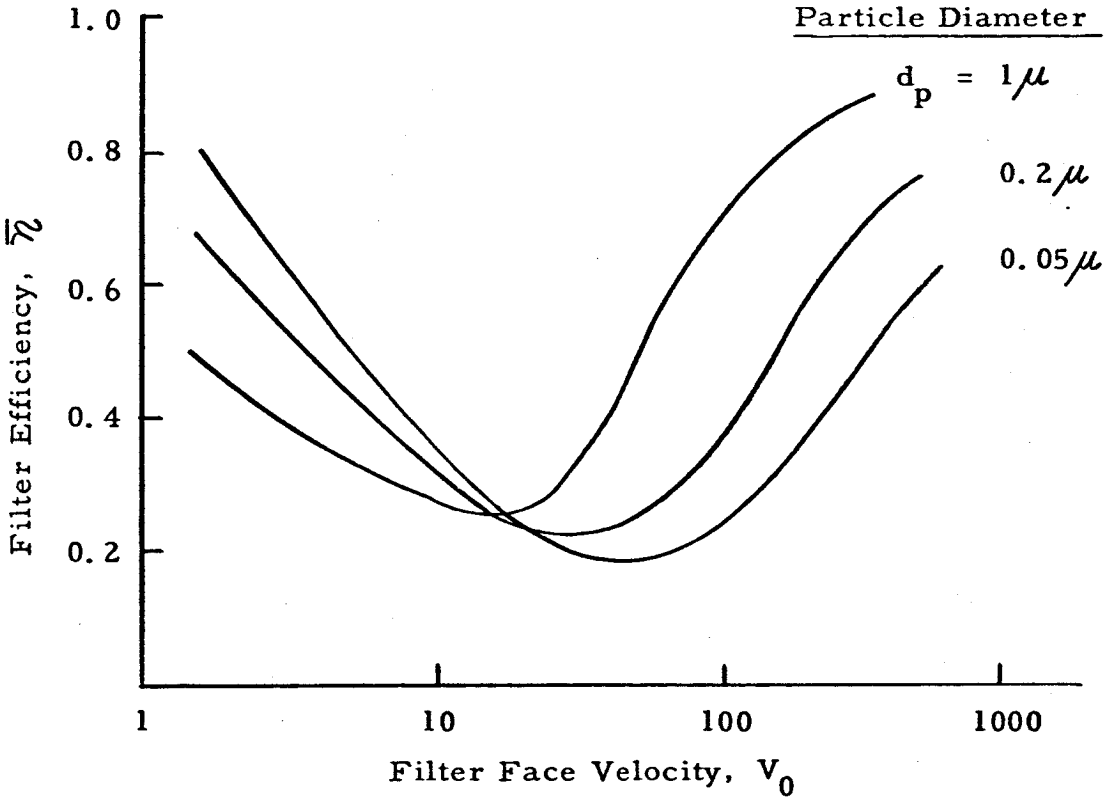


Figure C-5. A Typical Filter Collection Efficiency Curve

of the filter or by using a filter composed of smaller fibers. However, both of these means for increasing collection efficiency result in increased pressure drop.

In comparing the filter to the inertial impactor or electrostatic precipitator as a particle collector for the Mars Microscope, we find that the filter is subject to a decisive disadvantage: the difficulty of removing the collected material from the filter matrix for observation or processing. Washing or chemical dissolution of the filter cannot be entirely ruled out as a possibility for the microscope system; however, these measures are of doubtful compatibility with subsequent processing of the particles.

V. AIR MOVERS FOR AEROSOL SAMPLING

All types of aerosol sampling system require some means for inducing a flow of air through the particle collection stage. Two types of air movers have been considered for this purpose; the centrifugal blower and the ejector pump. The performance of these air movers is discussed briefly herein to establish a basis for estimating the size, weight and power requirements of aerosol sampling systems.

A. Centrifugal Blower

The requirements of a blower for collection of a sample of particulate material from the Martian atmosphere were indicated in the earlier discussion of an impactor particle collection stage. The power required for the impactor system can be estimated by the following analysis.

The adiabatic efficiency of a blower can be expressed by an equation of the form:

$$\eta \sim \frac{Q \Delta p}{w} \qquad \text{(C-5)}$$

where:

Q = the volumetric flow rate

Δp = the pressure increment developed by the blower

w = the power supplied to the blower

For the present purposes, an overall efficiency including both the electrical efficiency of the drive motor and the blower efficiency may be defined as follows:

$$\eta_e = 0.116 \frac{Q\, p_a}{w} \left(\frac{\Delta p}{p_a}\right)_b \qquad \text{(C-6)}$$

Units in this expression are: flow rate Q in cfm, power w in watts and p_a in inches of water. The dimensionless ratio $\left(\frac{\Delta p}{p_a}\right)_b$ is the pressure rise Δp across the blower divided by the ambient pressure p_a.

If a blower is used to induce flow through an impactor, the sampling flow rate as a function of power input to the blower is of fundamental importance in evaluating the practicability of the system. It was shown in Section IV. A that, for a fixed cutoff, the nozzle velocity and nozzle diameter of an impactor must be kept in a fixed ratio; i. e., from Equation (C-1):

$$\frac{V_n}{d_n} = \frac{18\mu\psi}{\rho_p C d_p^2} = \phi \qquad \text{(C-7)}$$

Assuming incompressible flow in the impactor, the nozzle velocity V_n can be expressed as:

$$V_n = \left\{2 R T_a \left(\frac{\Delta p}{p_a}\right)\right\}^{1/2} \qquad \text{(C-8)}$$

T_a being the absolute temperature. Using Equations (C-6) through (C-8) and noting that $Q = A_n V_n$ (A_n denotes the impactor nozzle area), we find that:

$$Q = 7.65 \left(\frac{\eta_e w}{p_a K}\right)^{0.6} \phi^{-0.8} \times 10^4 \qquad \text{(cfm)} \qquad \text{(C-9)}$$

The factor K appearing in this expression is a pressure loss factor which, when multiplied by the nozzle kinetic energy, gives the total pressure loss across the impactor. Experience indicates that K = 1.25 is a realistic value for a circular impactor. Using the values $\phi = 2.2 \times 10^4$ for a cutoff at 0.5 micron for particles of density $\rho_p = 1.0$ (Equation C-2) and $p_a \approx 37.3$ inches of water, we obtain from Equation (C-9):

$$Q = 2.7 (\eta_e w)^{0.6} \qquad \text{(cfm)} \qquad \text{(C-3)}$$

The corresponding "design" values of blower pressure ratio and impactor nozzle diameter are found to be:

$$\left(\frac{\Delta p}{p_a}\right)_b = 0.085 (\eta_e w)^{0.4} \qquad \text{(C-3)}$$

$$L_n = 0.465 (\eta_e w)^{0.2} \qquad \text{(cm)}$$

These results are used in Section IV. A to estimate the performance of an impactor aerosol sampling system. The overall efficiency η_e of the motor blower must be determined experimentally. In general, it can be expected that the efficiency will itself be a function of the flow rate Q.

B. Ejector Pump

The possibility of using an ejector pump for inducing flow through a particle collection stage has also been examined. In this application the ejector would be powered by a stored high-pressure gas supply. A sketch of the ejector configuration may be found in Figure C-6. The ejector functions essentially as follows: A high-speed primary gas stream is introduced into a mixing tube, where it entrains a secondary airstream through turbulent mixing and momentum transfer. The mixed stream is then exhausted to the atmosphere through a diffuser which converts most of the kinetic energy of the flowing gas into a static pressure rise.

The ejector pump is capable of developing a sufficiently high secondary pressure ratio, to satisfy the requirements of either filter or impactor particle collection stages. At the same time, the secondary-to-primary mass flow ratio W will generally be greater than unity; for example, $W \approx 2.0$ for a secondary pressure ratio of 1.25, increasing to $W \approx 3.0$ for a secondary pressure ratio of 1.15. In the present context, large values of W enable a large volumetric sample to be taken with the expenditure of a small mass of primary gas.

The calculated performance of an ejector system designed for a secondary pressure ratio of 1.25 is shown in Figure C-6. The specific design parameters indicated in the figure were specifically chosen for a descent sampling system (Figure C-7) where it is desirable to secure the highest possible particle concentration on the collection surface during a short exposure time. However, this sampling device could also be used for aerosol sampling at the surface of Mars. Under the latter conditions the sampling rate would be approximately 3 cfm and a volume of about 300 cubic feet could be sampled by expending 1 pound of primary gas. It will be noted that the device is quite small (the sketch is drawn approximately to scale).

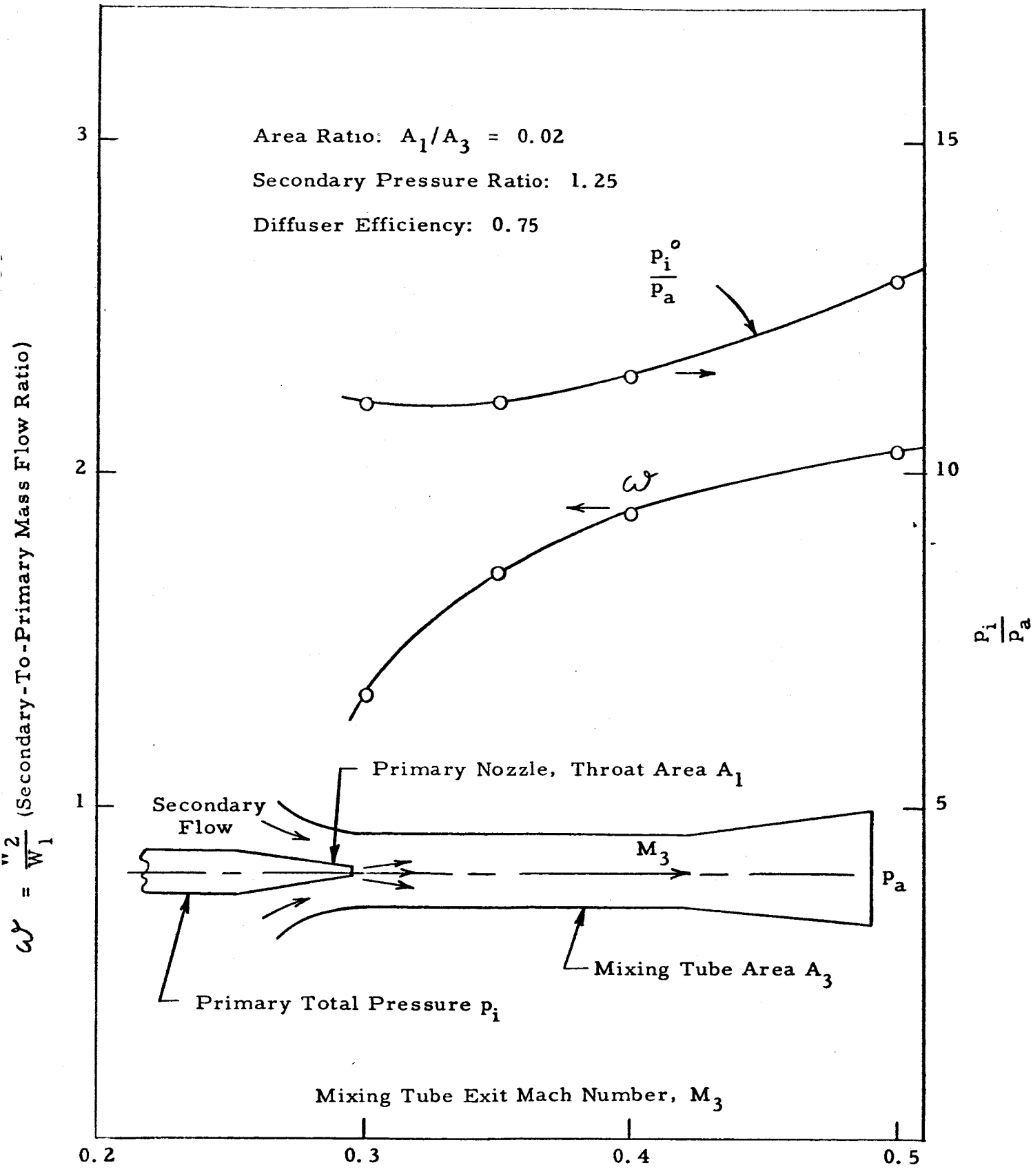
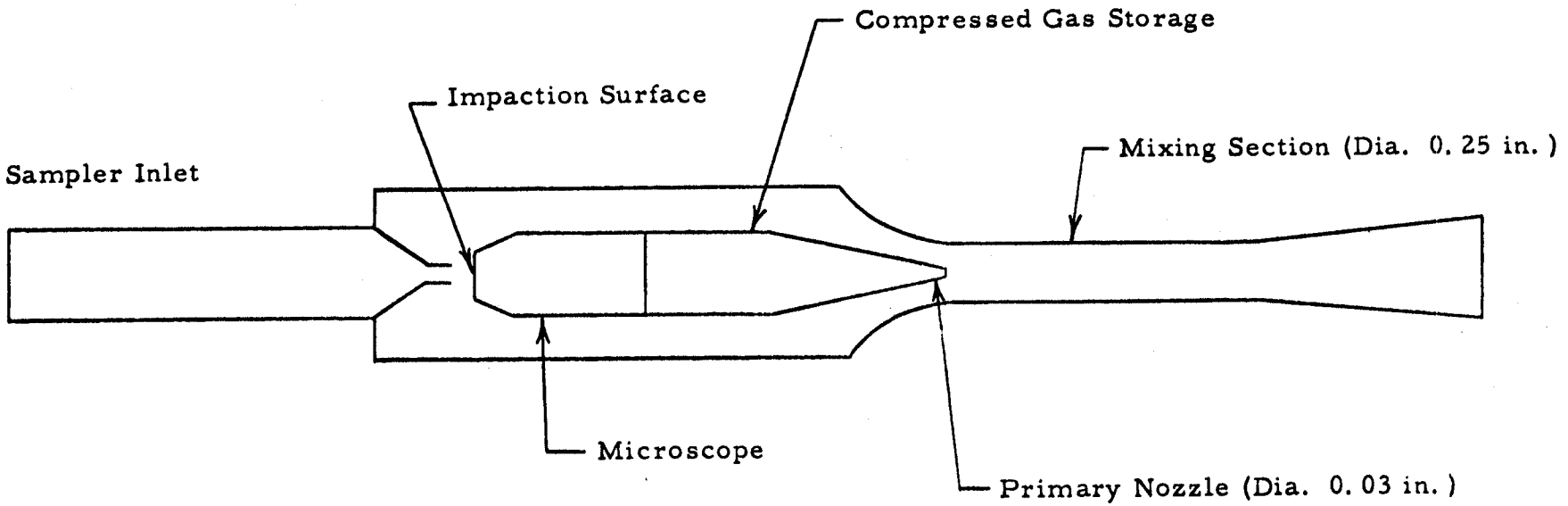


Figure C-6. Ejector Design Characteristics
(Convergent Nozzle, Constant Area Mixing)



Operating Conditions

Impactor:	Nozzle Mach Number	0.41
	Nozzle Diameter	3.5 mm
	Cut-off	0.5 μ (particle density 1.0)
Ejector:	Secondary Mass Flow	0.02 #/min
	Primary Mass Flow	0.01 #/min
	Secondary Pressure Ratio	1.25
	Primary Pressure Ratio	12.00 (referred to ambient)

Figure C-7. Schematic - High Frequency Descent Sampling Device

VI. SIEVING METHODS TO REMOVE EXTRA-LARGE PARTICLES DURING COLLECTION

Sieving has been considered for our application as a means of initial size separation during sample collection, i.e., to remove those extra-large particles which would interfere with the subsequent operation of the system. Standard sieves are of woven-wire cloth with uniformly spaced square openings; although silk and nylon cloth sieves are made, and sieve openings of rectangular or circular shape are also frequently used. Standard woven-wire sieves are available with openings in the size range from 37 microns to over one centimeter.

A special 15-micron woven-wire mesh is made in Germany. Other special electro-formed sieves are available with square openings covering the size range of 5 to 150 microns. These sieves are produced by a photo-engraving and electroplating technique by the Buckbee Meers Co. of St. Paul, Minnesota.

There are two basic approaches to size separation by sieving: the first is a steady state process which involves a constant and continuous feeding of the material to be separated; the second approach is a non-steady state process which involves sieving a given sample of material. The fractional quantity of material passing through a sieve is a rather complex function of material composition, quantity, particle shape, particle size distribution, sieve opening size and time.

The first stage of sieving is that during which particles much smaller than the sieve opening pass through. After a sufficient time, particles "much smaller than" will have essentially passed through, leaving particles of approximately the sieve size and larger remaining. Sieving in this second region is a probability function relationship which approaches completion with increasing time.

Steady state sieving is mainly in the first region, while non-steady state can be in either region if sufficient time is allowed. Normally, it is only necessary to allow sufficient time for the majority of particles in the size range of interest to pass through.

The size range over which sieving can be used for size separation is determined by the basic material to be sieved. Sieving of dry particulate matter results from gravity pulling particles through the sieve opening. If the opening is smaller than the particle, the particle cannot pass through. If spherical particles of approximately millimeter size are to be passed through a square sieve opening, the opening size is the important parameter. When particles of micron size are to be sieved, gravity force is no longer predominant over adhesive forces. Therefore, gravitational force is no longer capable of "pulling" a particle away from a surface with which it is in contact. Sieving is an inefficient method of size separation or classification of dry particulate matter below about 50 microns diameter.

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APPENDIX D

SAMPLE PROCESSING

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I. INTRODUCTION

The term sample processing includes those measures which are required to:

1) Remove the particles from the collecting substrate.

2) Produce the disadhesion of microorganisms from their mineral matrix.

3) Isolate the biological constituents by density fractionation from the remainder of the particle sample.

4) Divide the biological particles into selective size fractions.

5) Concentrate each size fraction within as small an area as possible on a suitable viewing surface.

Other optional aspects of sample processing include chemical techniques such as culturing and staining.

Items (1) and (2) constitute a preliminary, although quite important, aspect of the required processing routine. The need for Item (1) arises from the fact that a thin sticky coating is probably required for effective retention of particles on the impactor collection stage. Experimental studies will be necessary, however, to establish the best type of substrate coating in terms of its retentive properties and its dissolvability in the liquid which will be used to perform the subsequent density fractionation. Item (2) is required on the basis of our knowledge that a very large percentage of terrestrial microorganisms are "hitch-hikers" which adhere to mineral particles. Thus, some means for producing disadhesion of particle agglomerates will be required, either by means of a detergent additive in the processing liquid or by mechanical agitation. This aspect of preliminary processing also constitutes an important experimental study phase.

Item (3) is regarded as the most critical processing requirement since the typical number concentration of terrestrial microorganisms in soil and air is of the order of one per 10^4 mineral particles. Thus, without a very

effective method for isolating the biological particles, they would be masked out during the observation phase by the mineral particles, as has been clearly demonstrated by our laboratory experiments. Fortunately, it is possible to separate lower-density microorganisms from higher-density background particles by gravitational or centrifugal flotation in a liquid of intermediate density. As will be subsequently explained, the centrifuge approach offers an obvious advantage over the gravitational method since it can produce the required density fractionation far more rapidly.

The necessity for Item (4) arises from the fact that the separation of the biological sample into discrete size classes can permit major simplification in the microscope design. We have, therefore, examined quite closely the potential capabilities of filters and micro-mesh sieves for performing size fractionation and have concluded that an intermediate filter or sieve for separating out the 10 to 50-micron particles is feasible for our application.

Item (5) is viewed as an extremely important processing requirement, since concentration of the microbial samples onto viewing areas on the order of 10^3 - $10^5 \mu^2$ could eliminate the need for any horizontal scanning of the specimen slide. Each sample could be concentrated within an area approximately equivalent to the microscopic field-of-view at the required level of magnification. The three promising approaches which have evolved from this study include centrifugal impaction, micro-area filtration, and electrohydrodynamic precipitation. These three methods, which will subsequently be described in detail, all appear worthy of extensive experimental investigation.

II. DENSITY FRACTIONATION

By suspending a sample of particulate material in a liquid of appropriate density it is possible to float the low-density particles to the surface of the liquid while the heavier particles are removed by sedimentation.

For effective density separation, it is necessary to first break down the particulate debris composing the sample so as to put the light fraction in suspension. This requirement suggests that the flotation fluid should have relatively low viscosity and should also be compatible with suitable dispersing agents. It appears, that for example, a fluid such as ethelene glycol would be satisfactory for our application.

Natural flotation does not appear to be satisfactory for density separation since gravity sedimentation probably would not provide sufficient density discrimination. However, the desired discrimination could easily be obtained by centrifugation.

The settling velocity of a particle of diameter d_p and density ρ_p in a fluid of density ρ and viscosity μ can be determined from Stokes law, as follows:

$$v = (\rho_p - \rho) \frac{a\, d_p^2}{18\mu} \qquad \text{(D-1)}$$

where a is the local acceleration. For natural sedimentation at the surface of Mars, $a \approx 380$ cm/sec. If the density separation is carried out in a centrifuge, the acceleration is: $a = \omega^2 r$, where ω is the angular speed in rad/sec and r is the distance from the axis of rotation (neglecting gravity). The time for a particle to fall from radius r_1 to radius r_2 in the centrifuge can be found by integration of Equation (D-1):

$$t = \frac{18\mu}{(\rho_p - \rho)\omega^2 d_p^2} \ln\left(\frac{r_2}{r_1}\right) \qquad \text{(D-2)}$$

For purposes of illustration, suppose ethelene glycol is used as a flotation fluid ($\rho = 1.25$, $\mu = 10$ cp @ 20°C); assuming also that $r_2 = 1.5$ cm, $r_1 = 1.0$ cm, $\rho_p = 2.0$, $d_p = 1.0$ micron, and $\omega = 400\,\pi$ (12000 rpm), we

obtain t = 75 sec as the settling time. On the other hand, if it is desired to concentrate the low-density particles at the surface, we must determine the time required for a light particle to rise from r_2 to r_1. Assuming ρ_p = 1.20 for such a particle, we obtain t = 75 x 25 = 1875 sec (about 30 min). For a particle 0.5 micron in diameter, these times are increased by a factor of four.

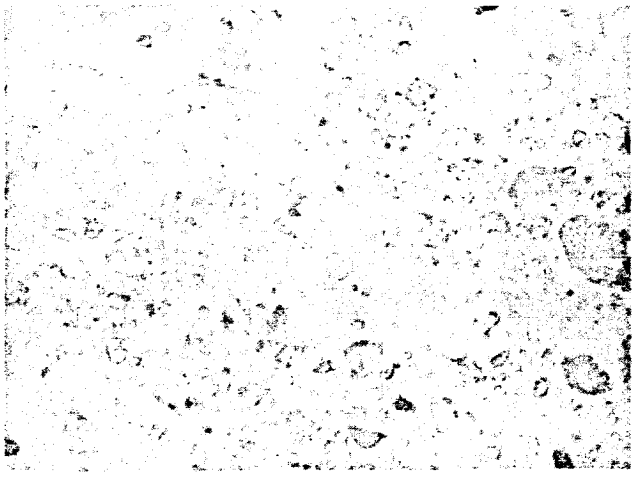
For gravitational settling, the sedimentation times given above are increased in the approximate ratio 4000:1. These calculations show that natural sedimentation is not feasible for density separation.

A list of potential flotation liquids is shown in Table D-1. It may be noted that there are certain inorganic and organic liquids (insoluble in water) which may be suitable for both density fractionation and for subsequent EHD concentration. Experimental studies will eventually be required to establish the ultimate feasibility of certain liquids as media for both processing methods.

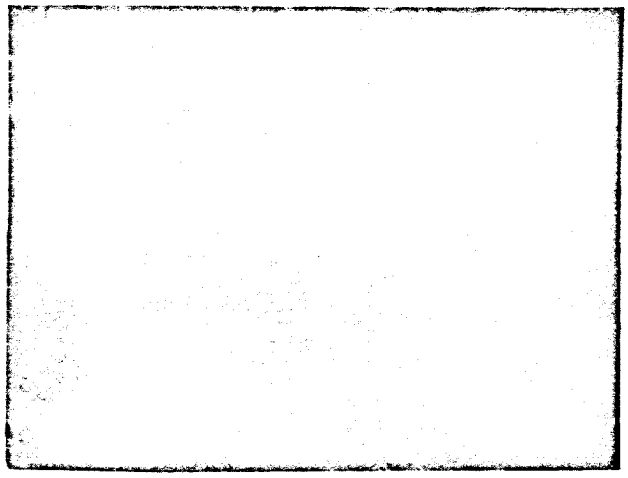
The feasibility of centrifugation as an effective means for density fractionation has been recently demonstrated by experiments with clay samples innoculated with A. niger spores. In Figure D-1 is shown a series of four photomicrographs illustrating these experiments. The photo on the upper right clearly points out the necessity for density fractionation of raw collected samples, because in this instance, even with an unusually heavy concentration of innoculated spores, the biological portion of the sample is thoroughly masked by the clay matrix. The two lower photographs, taken of the low-density fraction after centrifugation at 2000 rpm for 10 minutes in Ludox HS, indicate that the higher-density clay particles have been effectively separated from the spores.

Table D-1. Potential Flotation Media (Liquids)

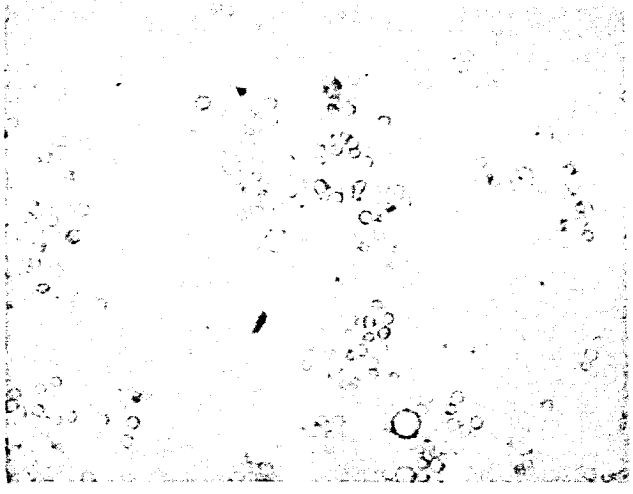
Material	Density	MP	BP	Water Solubility	Comments
1. Organic Liquids Compatible with Culturing					
Mercaptoacetic Acid	1.32	-16.5	104	s	Normal ingredient of nutrient media
Methyl Glycerate	1.28		239	∞	
Glycerol	1.26	FP < 0 17.9	290	∞	Extremely compatible
Glycerophosphoric Acid	1.59	-20		∞	Substrate in microbiological assays
Lactic Acid	1.25	18	122	∞	Substrate ingredient
Methyl Malate	1.22		242	vs	
α-chloropropionic Acid	1.28		186	∞	
Pyruvic Acid	1.267	13.6	165	∞	"Hub" of metabolic wheel
Ethyl L-Tartrate	1.20		162		
2. Industrial Chemicals, High Density, Water Soluble, Liquid					
Sorbitol	1.322			s	
Diethylene Glycol Diglycolate	1.30			∞	
Ethylene Chlorhydrin	1.20			∞	
Ethylene Glycol	1.25			∞	
Glycerin α Monochlorhydrin	1.32			∞	
3. Soluble Inorganics					
Ludox Series (DuPont - potassium silicates)					
4. Insoluble Inorganic Liquids (suitable for flotation and EHD concentration)					
Antimony Pentachloride					
Phosphorus Trichloride					
Silicon Tetrachloride					
5. Insoluble Organic Liquids (suitable for flotation and EHD concentration)					
Halocarbon Series (3M)					
Silicon Oils					



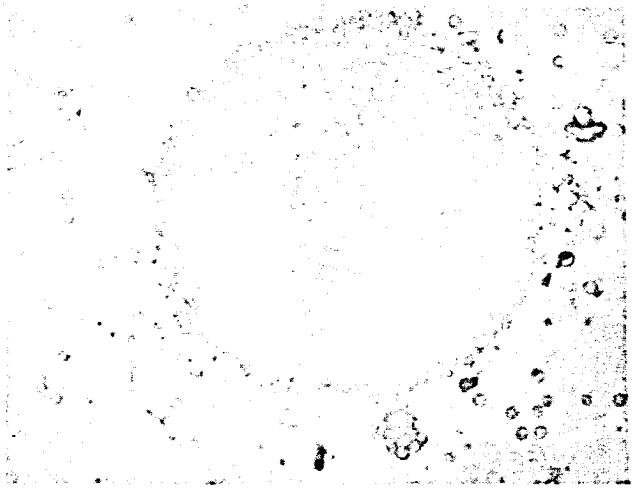
Clay Matrix (suspended in Ludox HS)



Clay Matrix plus approximately 10^6 spores/gm (suspended in Ludox HS). (Spore-clay mixture prepared by covering surface of mycelial mat of A. niger with clay and shaking thoroughly).



Concentration of spores at meniscus after centrifuging 5 gm clay-spore mixture in Ludox HS for 10 minutes at 2000 rpm.



Conidiophore and spores concentrated in meniscus. Note relative freedom of field from inorganic clay particles.

Figure D-1

III. SIZE FRACTIONATION

The term size fractionation is applied here in terms of further size division of a biological sample whose maximum and minimum size limits have already been established during the collection process. It has been previously shown that a reasonably sharp minimum cutoff size can be established for the collected particles by suitable design of the inertial impactor. Similarly, a dry sieve unit can be readily incorporated into the collection inlet to prevent the entry of extra-large particles. (As an option, a wet sieving process could be used to remove such particles from the fluid after the particle agglomerates have been reduced.)

The ultimate purpose of size fractionation is to enhance the observability of a biological specimen placed under the microscope. There appear to be significant design advantages in terms of simplifying the microscopic design. For example, if no size fractionation were employed and if all the microorganisms in the 0.5 to 50-micron size range were to be placed with a specimen slide area sufficiently large to allow for the possible presence of the largest particles (an area, say, of 100 to 200 microns in diameter), then horizontal scanning would be required to observe the smallest particles at the highest magnification mode (i.e., 900 to 1000X overall magnification) where the field-of-view is only some 18 to 20 microns in diameter. It has been our intent, therefore, to determine to what feasible extent size fractionation can be carried out. The ultimate aim was to possibly develop a system whereby separate microscopic examination could be performed on specimen slides which contained only those particles within a size range resolvable within the depth of field corresponding to one focus position for a given level of magnification.

Our studies have revealed that filtration (we shall use this term to also include micro-mesh sieving) is the only promising method for carrying out effective size fractionation of particles suspended in a liquid medium. (Another possible approach which is viewed as impractical for our application is to re-aerosolize the particles and to use a multiple-stage impactor

for size fractionation.) Further study has also shown, however, that the recovery ratio of the smallest particles can be seriously reduced by their successive passage through a series of filters used to collect out larger particles. This decreased recovery effect is quite significant for smaller pore size filters. For example, if we were to use 5.2 and 1-micron pore size filters in series (to collect particles in the 5 to 50, 2 to 5, and 1 to 2-micron size ranges) a significant portion of the 4 and 3-micron particles would be lost within the pores of the 5-micron filter and a large fraction of particles smaller than 2 microns would become embedded within the 2-micron filter.

Nevertheless, the use of one intermediate filtration step for size fractionation does appear feasible for our application if the filter pore size can be selected within an appropriate range to minimize losses of the smaller particles which pass through. A 10-micron pore size filter has been selected as the best compromise to collect out the larger particles for low-power observation and to pass the smaller 0.5 to 10-micron particles through to eventual collection on their viewing surface without any appreciable entrapment within the filter. It should, of course, be pointed out in this regard that filter surfaces are quite suitable for lower-power (e.g., 100X) observation of larger particles (where the depth of view is sufficiently large to tolerate local irregularities of up to several microns from the mean plane of the filter surface) but would pose greater problems for higher-power (e.g., 1000X) observation. The recommended design concept which we are proposing for the Mars Microscope uses a 10-micron filter for one-stage size fractionation. Experimental studies will be especially important in confirming the ultimate validity of this approach.

IV. CONCENTRATION

Without doubt, the concentration of the light-density sample fractions to within the smallest possible viewing areas is a vital problem area. Although certain preliminary experiments performed under this contract

have shown great promise, no definitive statements are yet possible on how effectively particles may be concentrated onto viewing areas which are on the order of 10^3 to $10^5 \mu^2$. The importance of effective concentration to simplified microscope design is quite obvious in terms of the horizontal scanning which is required: if we consider, as an example, a field-of-view at high power whose diameter is 20 microns, some 5000 fields would have to be scanned to effectively analyze a sample contained within a 1 mm^2 area. If we further consider the implications of proposed microspectrophotometric scanning at intervals of 1 micron or less within each 20-micron diameter field to detect the possible U.V. absorption characteristics of individual particles, it is obvious that a very high degree of particle concentration on the viewing surface must be achieved.

The three possible approaches to the problem of highly concentrating the light-density particles presently appear to be 1) centrifugal impaction, 2) micro-area filtration, and 3) electrohydrodynamic precipitation. Each of these methods will now be discussed in some detail.

A. Centrifugal Impaction

The centrifugal impaction method was actually conceived as an extension of the design concept for performing the density fractionation. After the density separation has been accomplished in the centrifuge chamber, the problem remained as to how best to remove light-density material from the supernate (and to concentrate it upon a suitable viewing surface). These objectives can be attained in a very simple way by the arrangement shown in Figure D-2, and can best be discussed in terms of the entire process beginning with the density fractionation.

The flotation fluid containing the particulate sample in suspension is first introduced into a centrifuge chamber as shown in the figure. A plastic sac containing a gas is provided at the outer end of the centrifuge chamber. When the centrifuge is brought up to speed (e.g., 18000 rpm), this sac is

compressed to a small fraction of its initial volume. This causes the fluid level to drop to the level "S" on the diagram. A valve operated by centrifugal force opens the orifice "0" to a second centrifuge chamber adjacent to the main chamber when the rotational speed reaches an appropriate value. After centrifugation for about 10 to 20 minutes, the speed is reduced to a preselected value, allowing the sac to expand so as to drive the surface layer of the supernate into the adjacent chamber.

With proper design, the particulate material of interest for microscopic examination will be contained within the fluid transferred to the second chamber. To present this material for microscopic examination, it is necessary to concentrate it on an optical surface. In principle this could be done either by passing the fluid through a suitable micropore filter of small area or by centrifuging again in a light fluid (e.g., $\rho = 0.8$) so as to concentrate the material on a microscope slide placed at the outer end of the centrifuge (see Figure D-2). The latter approach, i.e., centrifugal impaction, does not appear to be impracticable at this time. It is anticipated that use of a filter for high magnification microscopy would be difficult, if not actually impossible, from an optical standpoint. In any case, filtration offers no apparent advantages over the differential centrifugation concept.

On the other hand, if it is desired to remove relatively large particles (e.g., $d_p > 10\mu$) for microscopic examination at medium or low magnification, a filter could be advantageously employed as shown in the figure. It will be noted that the filter would not function in the usual manner, however, since the fluid is stationary with respect to the filter. With a properly chosen filter, a sieving action will occur with a negligible loss of small particles since a strong settling potential will exist due to centrifugation.

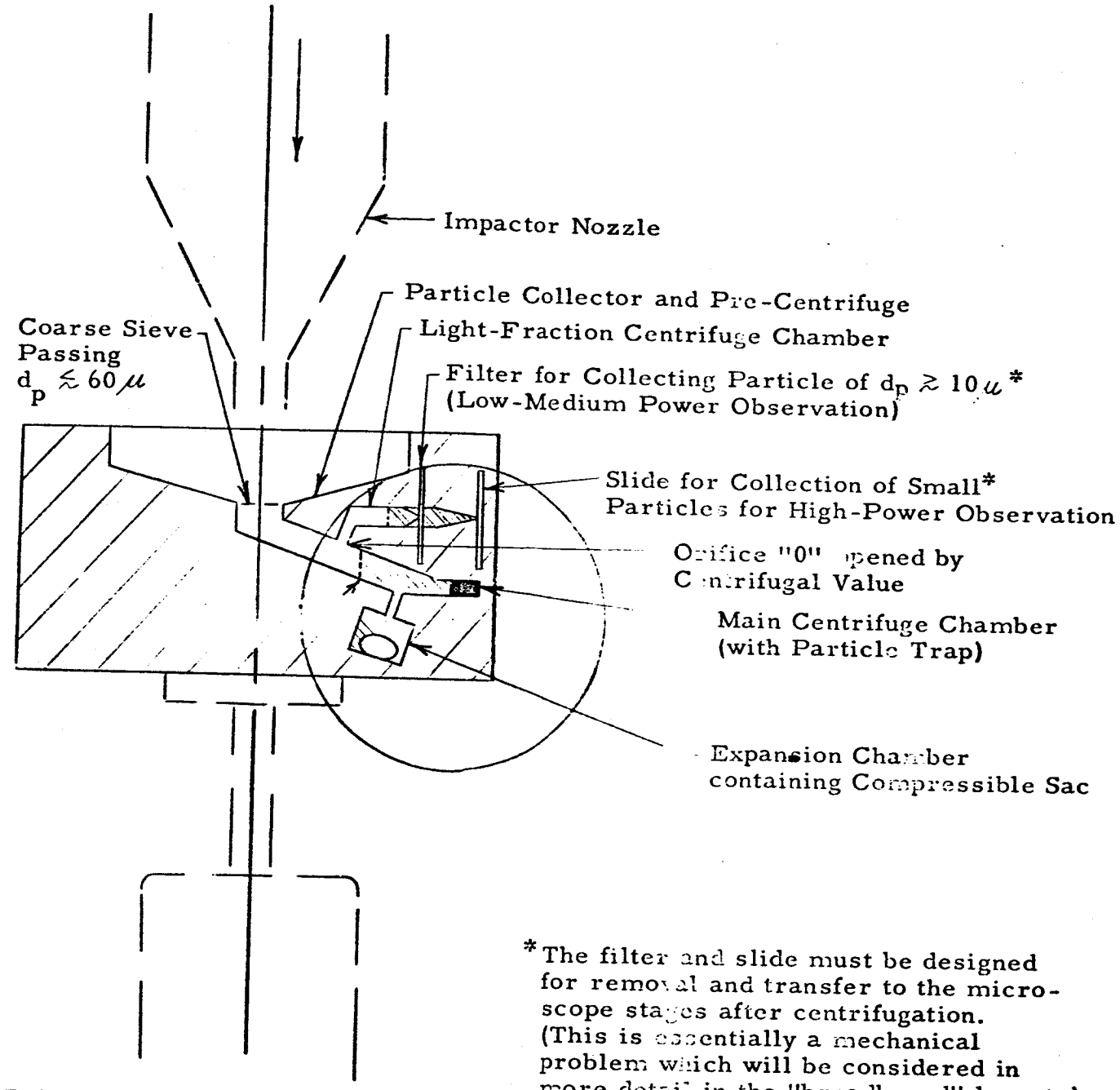


Figure D-2. Schematic of Differential Centrifugation Device for Sample Processing

B. Micro-Area Filtration

As the idea of micro-area filtration was originally conceived, it involved a cone-shaped system of filters in series which collected each smaller size range of particles within progressively smaller areas. This concept evolved as a natural extension of our filter studies for size fractionation. Unfortunately, further investigation has revealed that there are certain limitations upon the number of serial filtrations that can be performed (if appreciable losses in the number of smallest particles were to be prevented) and that the microscopic observation of small filter-collected particles at high magnification has serious limitations. Nevertheless, the use of an intermediate filter for collecting out the larger 10 to 50-micron particles does appear feasible and does provide advantages in the microscope design. The problem can, therefore, be restated as one which principally involves the use of filters in a diameter range of 100 to 200 microns for collecting out the larger particles.

The use of filtration for concentrated particle concentration has only been generally applied for filters in the area range of 1 cm^2 or larger, although certain experimental studies have been performed with filter areas of approximately 1 mm^2. Nevertheless, there are no theoretical barriers to performing micro-area filtration upon areas as small as 100 microns diameter (or less). Of course, the relationship of liquid volume to filter pore size must be kept within practical limits if the liquid suspension is to be pushed or pulled through the filter within a reasonable amount of time and with minimal expenditure of power.

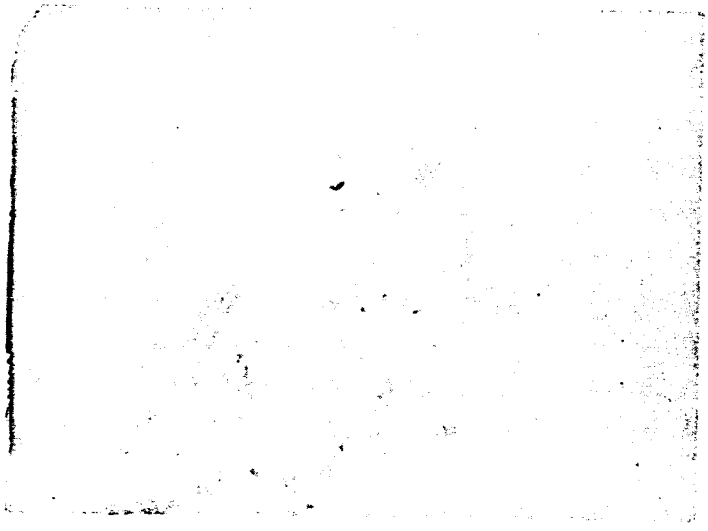
A simple experiment was devised to demonstrate the feasibility of micro-area filters for our application. A hole 340 microns in diameter was drilled through two metal plates, between which was then placed an ordinary Millipore filter (the size limit on the hole was determined by the smallest metal drill which happened to be immediately available). Known concentrations of biological specimens in aqueous suspension were then

aspirated through the filter, which was then removed from the metal plates and observed microscopically. The results, which are shown by the photomicrographs in Figure D-3, demonstrate that the collected material was indeed concentrated within an area equivalent to the hole size drilled in the metal plates, with little or no obvious signs of outside lateral spreading. These results seem to justify further experimental work with even smaller collection areas to determine quantitatively the merits of this approach.

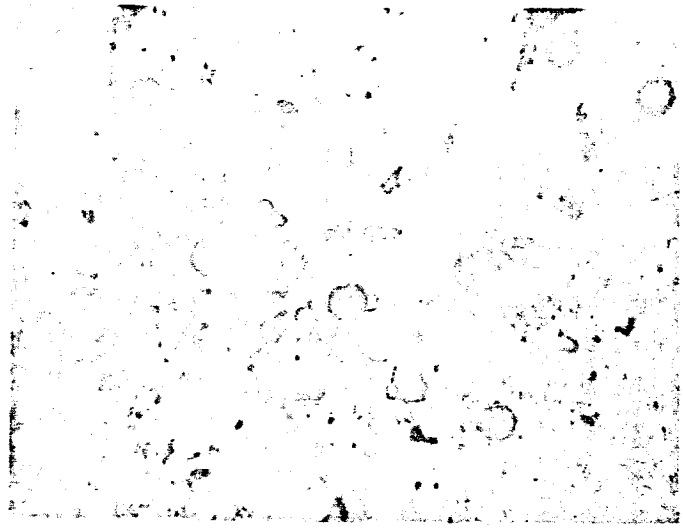
C. Electrohydrodynamic Precipitation

A concentrated precipitation of particles in a dielectric liquid medium can be viewed directly with a degree of success on a conducting surface of less than 1.0 mm diameter using an electrohydrodynamic (EHD) device. Several such electrohydrodynamic devices have been described in the literature by O. M. Stuetzer.

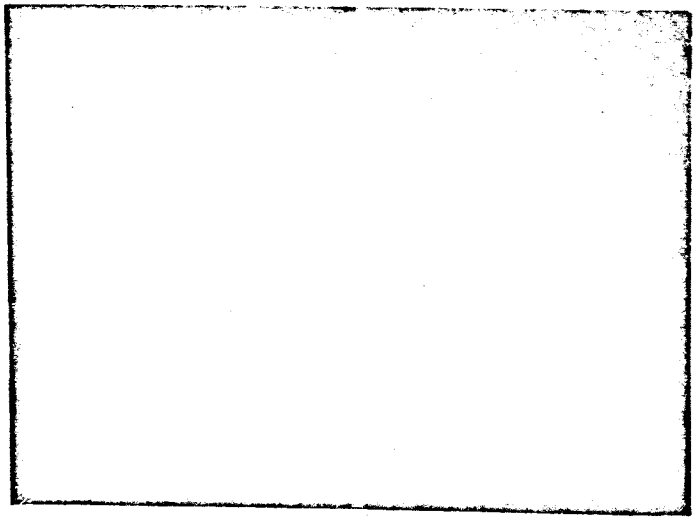
In our particle concentrator a high unidirectional potential and a current flow is set up between a corona point and a collector between which the liquid dispersion to be treated is passed. The liquid dispersion of particles flows through a strong field in which many ions are formed. It is imperative that a high enough field strength be maintained to create ionization as nothing will be collected that is not ionized. It is furthermore important, that the ions present are all of the same sign. As an ionized particle moves in the field, it may form an aggregate with a number of ions of like sign and be deposited on the surface of the collector. In aggregating with a number of ions of like sign, the cluster will retain the same electrical driving force per ion per charge in the electric field as if each ion where wholly independent, but the resistance to motion would be reduced in accordance with Stokes' law. The volume of such a particle aggregate is approximately the sum of the large surface active ions, and thus the resistance to movement is proportional to the radius of the aggregate.



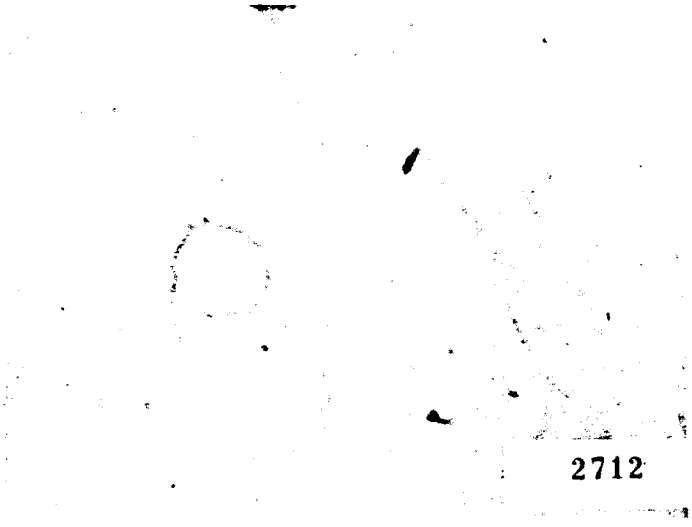
1. Material Concentrated on a 340μ Diameter Spot ($\sim$175 mag.)



2. Spot from Center of 1 Showing Mold Spore (Aspergillus niger) ($\sim$1700 mag.)



3. Serratia marcescens ($\sim$1700 mag.)



4. Conidiophore ($\sim$1700 mag.)

Figure D-3. Photomicrographs of Biological Specimens after Cencentrated Collection on Millipore Filter Surface 340 Microns in Diameter

Essentially our system proceeds as follows:

1) Particles are charged by means of electrons or ions.

2) Charged particles are transported through the dielectric liquid and precipitated at the collector by the force exerted on the charged particles by the electric field.

3) Particles are retained on the collector, either for viewing from outside the electrohydrodynamic device, or to remove the whole collector while maintaining the field.

4) Particles are removed by the field running off thereby preparing the collector for subsequent collections.

The charge a particle acquires is proportional to the field strength and the particle surface area. Theoretically, larger particles receive a greater charge and should therefore be precipitated more rapidly than small particles at the point of highest field strength.

Figure D-4 illustrates the experimental EHD biological particle precipitator which has been used during this phase of the feasibility study. This experimental configuration also shows an observing microscope and an oblique illuminator to achieve a dark field effect; this unit was used for direct viewing studies of the concentrated particles. Satisfactory qualitative results have been achieved using 1.0, 4.0, 4.8, and 6.4 mm diameter electrodes for collection of S. marcescens. Further theoretical and experimental work is required, however, to establish the quantitative collection efficiency and particle size classifications of this electrohydrodynamic device.

V. CHEMICAL PROCESSING OF BIOLOGICALS

Among the major biological problems anticipated in the Martian probe are the following:

1) Collecting sufficient biological material to yield a good probability of detection by microscopic means

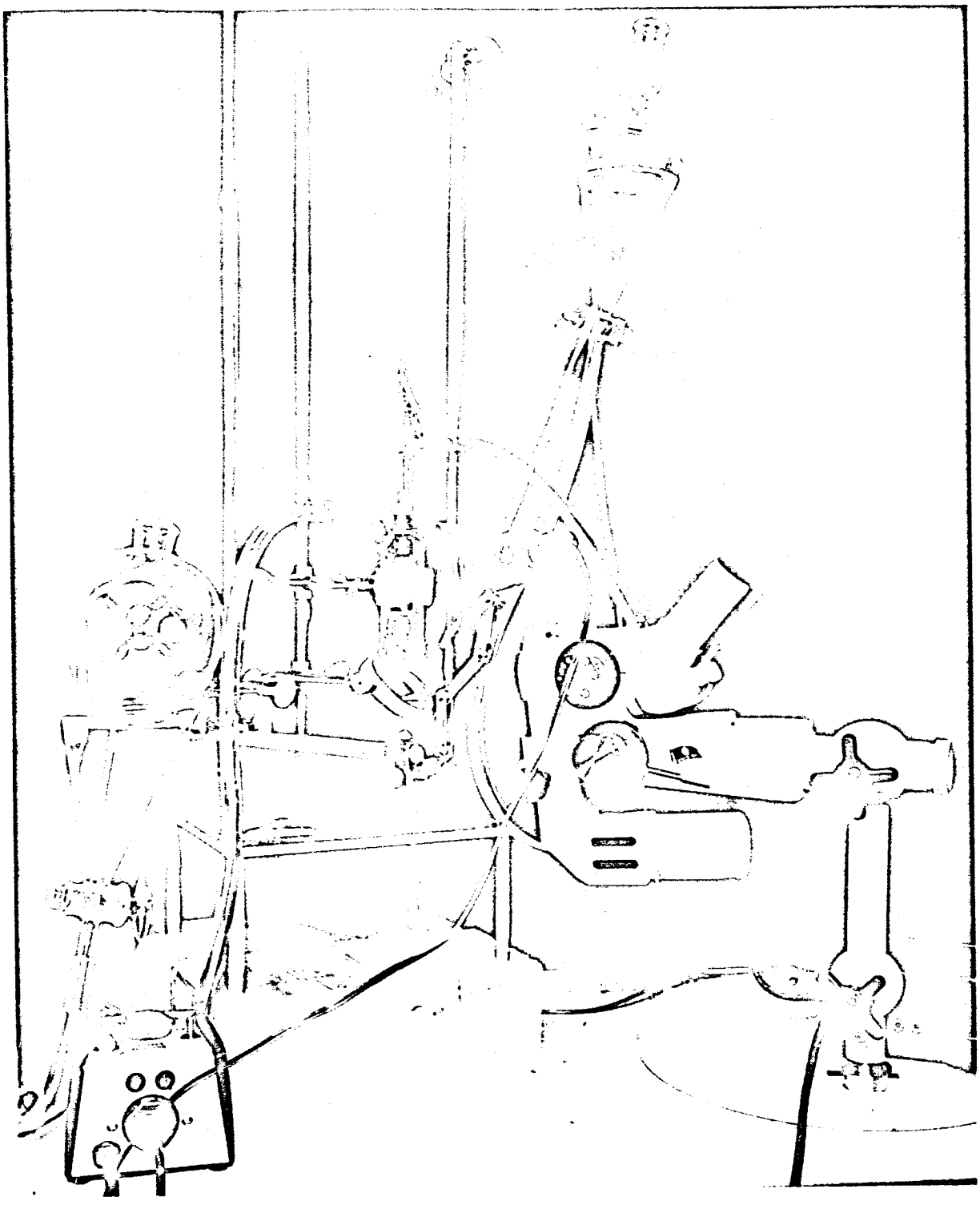


Figure D-4

Experimental EHD Biological Particle Precipitator and Dark Field Microscope for Direct Viewing of Concentrated Particles

2) Masking of the biological material by inert matter of the same size and shape

3) Detecting transparent cells of unknown chemistry by light microscopy

In order to mitigate these difficulties, some consideration might be given to chemical treatment of the collected aerosols, always keeping in mind that all means employed are directed toward the enhancement of microscopic detection of variable organisms.

An ideal chemical treatment would permit an accelerated proliferation of living cells, would intensify and delineate the optical appearance of any internal structures, and would concurrently dissolve the non-viable material which might interfere with the view. Unfortunately, such an ideal chemical treatment is not yet within the state of the art, nor is there any immediate prospect of its development. However, several techniques, though less sophisticated, might be considered as feasible.

A. Culturing

It is obvious that any culturing technique which encourages proliferation will improve the probability of detection of viable entities simply because these will be more to detect. Less obvious, but of equal importance, is the recognition that culturing might permit the development of cell clumps and microstructures not always present in the resting phase. The observation of structures such as nuclei, spores, capsules, conidia, rhizoids, branches, etc. will make verification of biological origin much more positive than the simple observation of undifferentiated cells. For example, the detection of coenocytic mycelium, or a blastospore, or a chain of cocci is worth more than the detection of simple mycelial fragments or cysts or individual cocci.

Culturing techniques compatible with th microscope system might be developed along the following lines:

1) The impactor collecting surface might be made from a dehydrated soluble nutrient mixture which will, upon contact with an aqueous flotation medium, serve as a source of energy and amino acids.

2) The flotation medium itself can be a nutrient solution. Several suitable liquids from the aspect of density and nutrition are: glutathione, pyruvic acid, lactic acid, and glycerol.

3) The "normal environment" (i.e., Martian dust) that will be collected along with the microorganisms might serve as enrichment media. This technique is used commonly by the students of petroleum microbiology, who grow their isolates in a mixture of fluid, sugar and environmental rock and tar.

Essentially, the enrichment procedure will require an incubation period after collection at a temperature close to the maximum encountered during the Martian day ($\sim$30°C). This requirement can easily be met.

B. Staining

If light microscopy is employed, detection will be facilitated if microbes are contrasted against their background. A technique developed by Ehrlich (J. Bacterial 70: 265, 1955) might be amplified and automated. Essentially, a pre-stained membrane filter can be employed and a dye added to the flotation medium. It is certainly within the state of the art to develop a suitable procedure with pure cultures. Further work will be necessary, however, to develop a biologically specific system in which the inert background contamination might be overwhelming.

APPENDIX E

MICROSCOPIC OBSERVATION AND DETECTION

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I. INTRODUCTION

The material in this Appendix is presented in an attempt to enumerate the most significant problems inherent in the design of the Mars Microscope optical system, and to suggest possible solutions. The material is meant to be inclusive but not completely definitive, in that the solutions described are treated only to the extent necessary to establish their possible feasibility or unfeasibility. Some parameters, such as the sample carrier (glass slide, tape, fixed electrohydrodynamic collection chamber, sample chamber integral with the centrifuge, etc.) are not treated, in that their selection will be determined largely by the choice of processing methods.

An attempt is made first to cover the theoretical factors which will help establish the design criteria, not only for the optical system but for the collection and processing systems as well. In particular, the criteria for design compromises are noted when possible. Secondly, the material suggests specific instrumentation which might well be incorporated into the design of the optical system, with the realization that some of the components discussed would require a concentrated development effort, even though they are in principle state-of-the-art.

II. FACTORS AFFECTING THE OPTICAL DESIGN

A. Resolution versus Focus, N.A., and Wavelength

The resolution achievable with a particular microscope is a function of many variables some of which are inherent in the theory of image formation and others which arise in the process of translating the theory into practice. The primarily theoretical variables include:

1) The numerical aperture (N.A.) of the objective

2) The numerical aperture of the condenser

3) The wavelength of light which is used

4) The obscuration ratio of the aperture, which is inherent in most reflecting objective designs

5) The distance of the object from the object plane (focus)

6) The index of refraction of the immersion fluid for the wavelength of light used

The variables affecting resolution which are of a more practical nature are:

7) The degree to which the aberrations are corrected in the optical design

8) The degree of perfection with which the optics are manufactured

9) The alignment of the optics, including the light source

10) Proper cover glass thickness and index

11) Proper tube length or object-to-image distance

12) The cleanliness of the optical surfaces and the immersion fluid

All of these parameters must be considered carefully when the best resolution is desired. The present discussion, however, concerns itself primarily with resolution as a function of focus, N.A., and wavelength.

The following definitions are used throughout this discussion (they also appear in Table E-1, page E-14, for easy reference):

z = resolution (in microns)
the minimum distance by which two objects can be separated and still provide resolved images
- also -
the distance in the object space corresponding to the radius of the blur circle in the image

z_{min} = the best resolution (in microns) theoretically obtainable for a given wavelength and numerical aperture

d = distance (in microns) from exact focus, or distance of the object from the object plane

d_{max} = focus tolerance (in microns), or maximum distance from exact focus that can provide best theoretical resolution

D = depth of field (in microns), the total range of focus in the object space for which the best theoretical resolution can be achieved; $D = 2\, d_{max}$.

λ = wavelength (in microns)

N. A. = numerical aperture of objective and condenser

n = index of refraction of immersion fluid for the wavelength used (for dry objectives, n = 1 for all wavelengths.

It can be shown* that when diffraction effects are neglected, the relationship between resolution and focus is given by the equation

$$z = d \frac{\text{N. A.}}{\sqrt{n^2 - \text{N. A.}^2}} \qquad \text{(E-1)}$$

This shows that when a particular N. A. and immersion fluid index are chosen, the plot of resolution versus focus is a straight slanting line which passes through the origin. This would indicate that at the exact focus (d = 0), the resolution would also be zero (infinitely good). However, from diffraction theory (page 497 ff of reference*) it is known that the resolution is limited by the wavelength of light used and by the numerical aperture of the objective and condenser according to the equation

$$z_{min} = \frac{\lambda}{2\ \text{N. A.}} \qquad \text{(E-2)}$$

* Hardy and Perrin. The principles of optics. New York, McGraw-Hill, 1932. pp. 77, 461, 510 ff.

This means that even at the exact focus, the resolution is finite. If this equation is plotted on a graph of resolution versus focus, the plot is a straight line for the constant value of z_{min}. The combined effect of the two equations when plotted on a graph of resolution versus focus becomes a slanting line converging toward the origin until it reaches the value of minimum resolution, where it turns and goes along that line. Figure E-1 illustrates this. Note the line marked N.A. = 0.5 DRY. This slanting line gives the value of resolution versus focus according to Equation (E-1). This line holds for all wavelengths because for a dry objective, n is always unity. However, the line is only valid for values of z which are equal to or greater than z_{min}. At the point (A), for example, the curve turns left to indicate that for visible light ($\lambda = 0.546\mu$), as d approaches zero, the resolution remains constant according to Equation (E-2). Similarly for U.V. ($\lambda = 0.365\mu$) the sloping line is valid down to point (B), where the horizontal line takes over. The points (A) and (B) represent the maximum values for d that will allow best resolution for the respective wavelengths. This value is obtained by substituting the value of z_{min} from Equation (E-2) into Equation (E-1) to give:

$$d_{max} = \frac{\lambda \sqrt{n^2 - N.A.^2}}{2\,N.A.^2} \qquad \text{(E-3)}$$

This represents the maximum distance from exact focus that will still permit the best resolution theoretically obtainable. The depth of field D, which is discussed later, is twice this value, or the total range of focus permitting best resolution, where:

$$D = 2\,d_{max} = \frac{\lambda \sqrt{n^2 - N.A.^2}}{N.A.^2} \qquad \text{(E-4)}$$

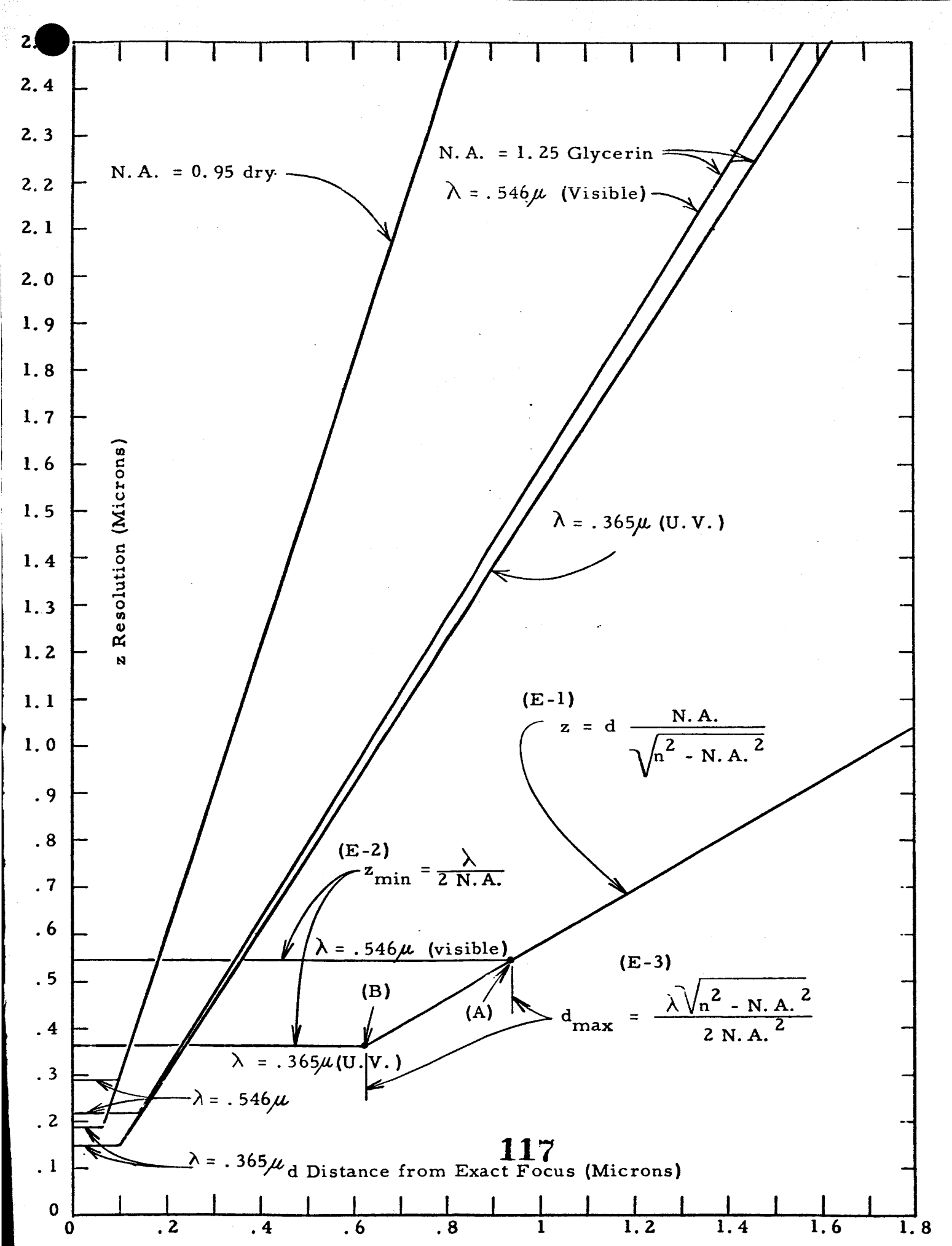


Figure E-1. Resolution versus Focus

Figure E-1 also shows the resolution versus focus curve for a dry objective of N. A. = 0. 95. This represents the practical limit for dry objectives and indicates how the good resolution is very dependent on sharp focus.

Figure E-1 also shows two curves for a glycerin immersion objective of N. A. = 1. 25. Two sloping lines are necessary because the index of the immersion fluid as found in Equation (E-1) is a function of wavelength. These curves indicate that using immersion objectives provides better resolution (because of higher numerical apertures) and also less sensitivity to focus error than dry objectives.

In the discussion thus far, it has been assumed that the curves in Figure E-1 could be derived solely from the equations given. To a close approximation this is true. However, because of the interaction between the diffraction effects and the geometrical effects, strictly speaking the curves are not composed of two straight segments. They are continuous lines, beginning at z_{min} for d = 0, rising slowly till they cross d_{max} slightly above the point (A) for example, and becoming essentially asymptotic with the slanting straight line. A rigorous treatment of this situation would be out of place here, but two statements should be made. First, for an object out of focus by an amount d_{max}, the resolution will not be quite as good as that plotted, so for the ultimate resolution, the microscope should be focused as carefully as possible. Secondly, the approximate curves shown are adequate considering the subjective nature of the criterion for resolution.

Figures E-2, E-3, and E-4 are plots of resolution versus focus for several different conditions. Figure E-2 is for dry objectives when used in visible light (0. 546μ) and U. V. (0. 365μ). Figure E-3 is for visible light immersion objectives where n = 1. 517. Figure E-4 is for U. V. glycerin immersion objectives where n = 1. 490 at λ= 0. 365μ, the plots for 1. 25 N. A. and 0. 85 N. A. corresponding to available objectives. If other immersion fluids, numerical apertures, or wavelengths are used, separate

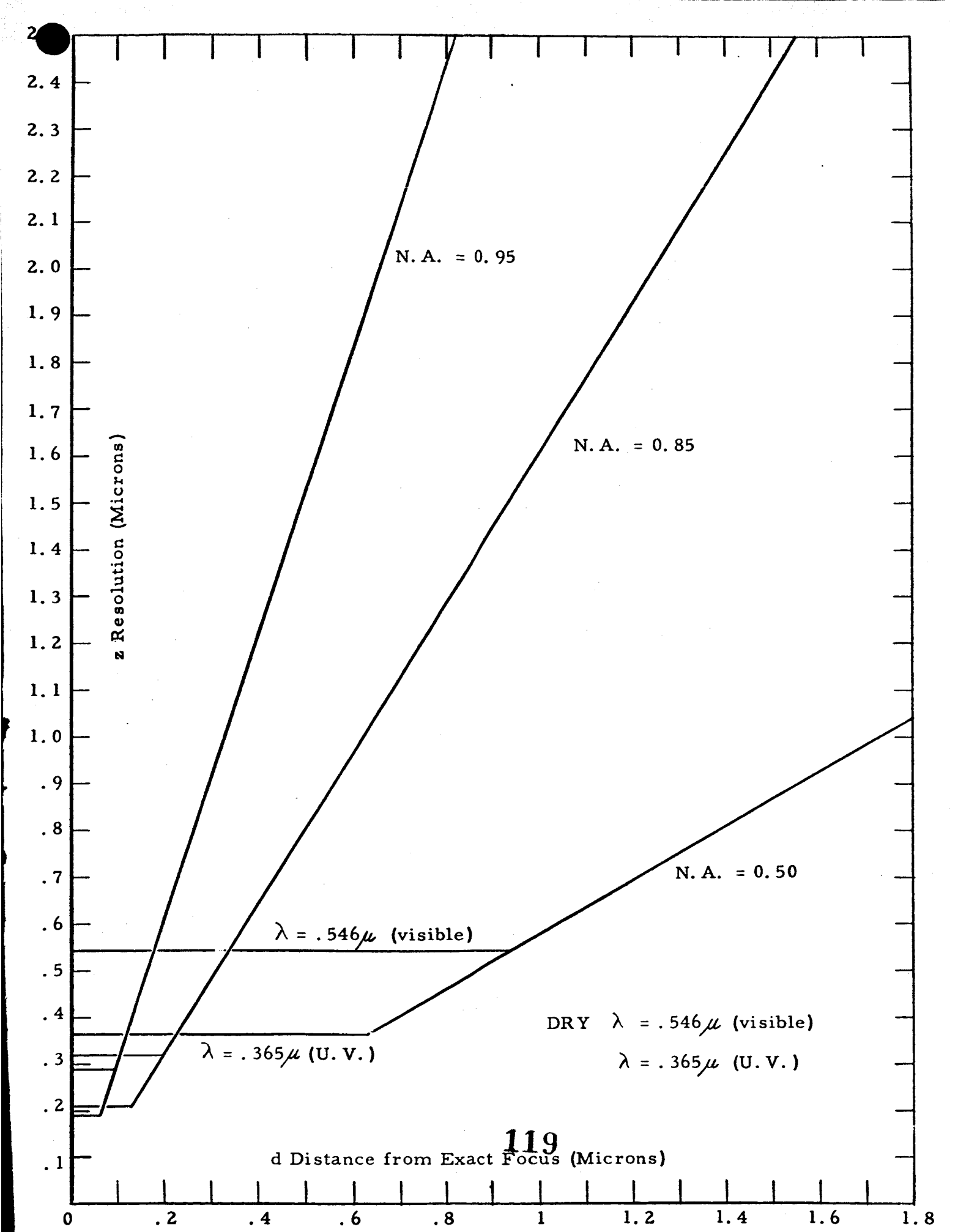


Figure E-2. Resolution versus Focus

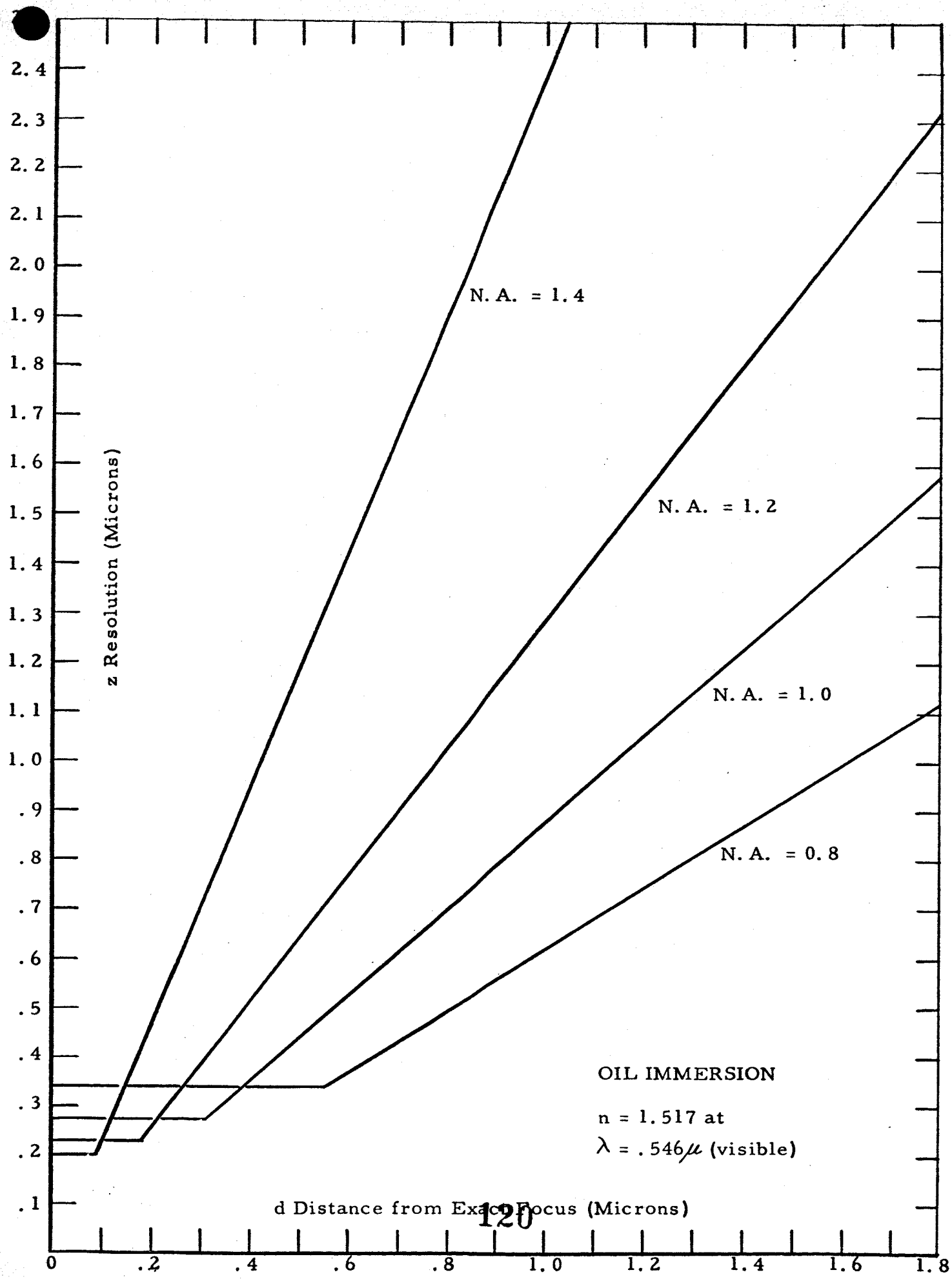


Figure E-3. Resolution versus Focus

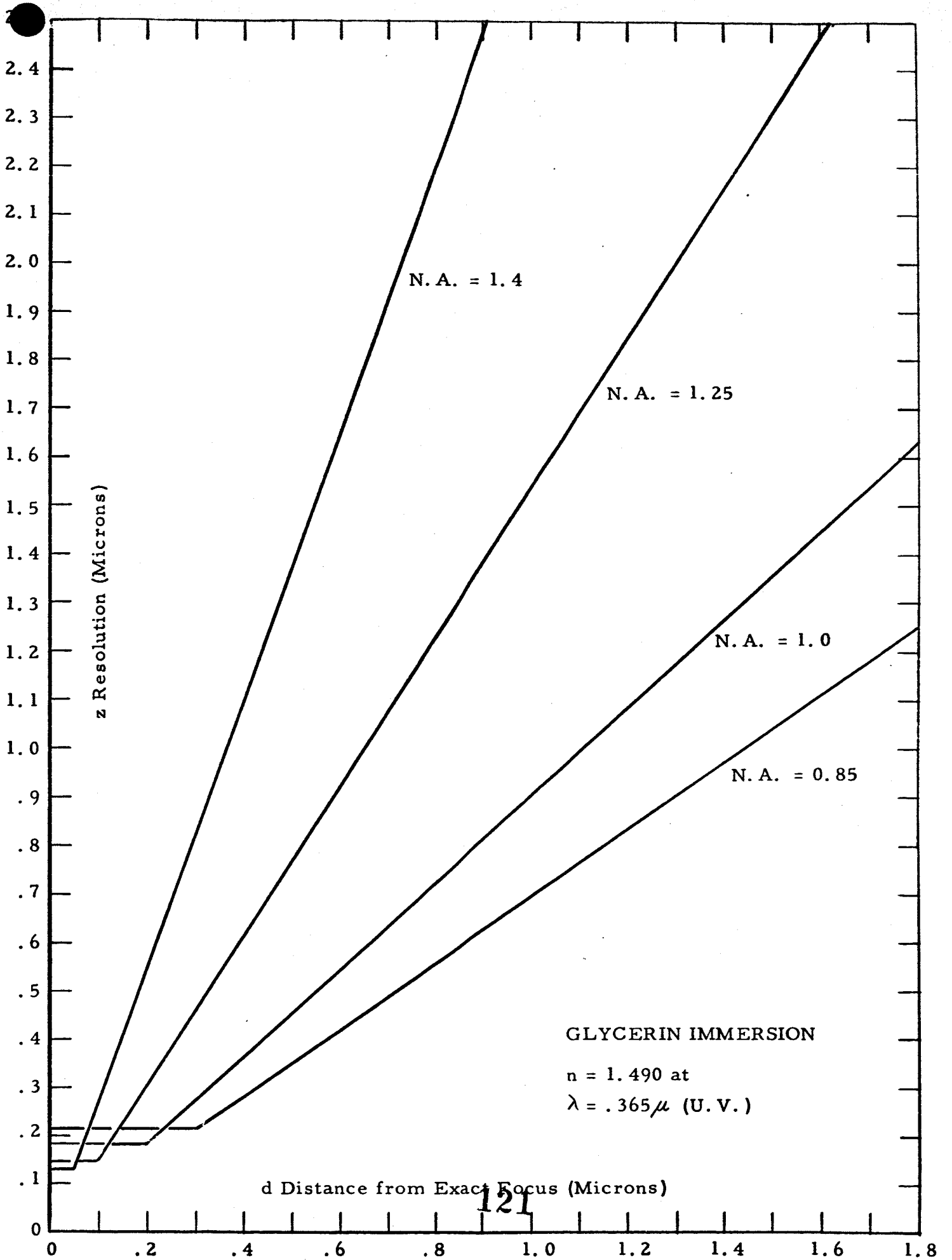


Figure E-4. Resolution versus Focus

curves can be calculated from the equations given and as illustrated in Figure E-1. However, in each case, one must use the proper index for the wavelength to be used.

An important consideration in microscopy is well illustrated in Figure E-3, for example. As the numerical aperture gets higher, the minimum resolution gets better (smaller), but the maximum distance from focus that allows best resolution gets smaller at a much faster rate, and the rate of degradation of resolution (slope of inclined curves) increases more rapidly. This means that the resolution inherent in high numerical apertures is more critically dependent upon proper focus than the even slightly degraded resolution given by a slightly lower numerical aperture. In other words, the higher numerical apertures should not be used in an attempt to achieve high resolution unless the focus error can be kept below d_{max}.

B. A Chart for Selecting Microscope Objectives

The performance of a microscope is a function of many variables as indicated previously. A few of these variables have been used to create a chart which can be useful in selecting microscope objectives (see Figure E-5). In the following explanation of how the chart was derived and how it is to be used, some general assumptions were made:

1) Highest quality optics are used and are aligned and adjusted properly. This includes the illuminator or light source.

2) The objectives are used in the manner for which they were designed, according to the best standards of practice.

3) The microscope is focused critically, at least within its depth of field.

4) The N.A. of the objective equals the N.A. of the condenser.

5) The obscuration ratio inherent in most reflecting objectives is not considered.

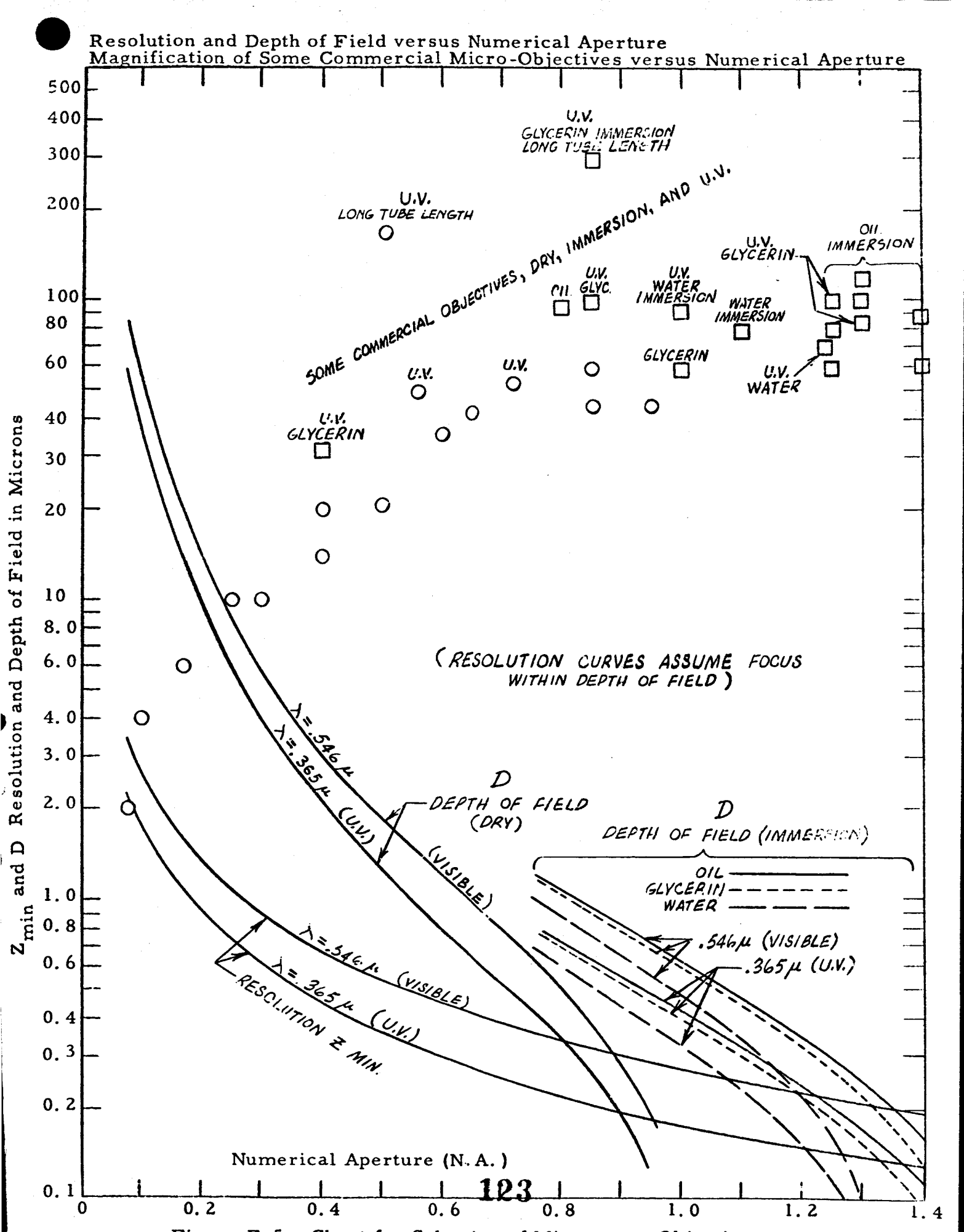


Figure E-5. Chart for Selection of Microscope Objectives

6) There is no image degradation due to any of the variables listed previously but which do not appear in the equations.

7) The definitions and equations previously given are adhered to throughout and should be consulted when using the chart. (See Table E-1.)

1. Resolution versus Numerical Aperture

The curves marked "Resolution z_{min}" are plots of the minimum separation in microns of two objects that can be resolved for a given N. A. and wavelength as computed from Equation (E-2). The equation is plotted for two wavelengths, 0. 546 micron in the visible spectrum and 0. 365 micron in the ultraviolet. Resolution for other values of N. A. and wavelength can be easily calculated from the equation. (If the microscope is not in focus within the depth field, D, the resolution must be computed from Equation (E-1) where resolution is a function of the distance from exact focus, the N. A., and the index of refraction of the immersion fluid.)

2. Depth of Field versus Numerical Aperture

The curves marked "D Depth of field" are plots of the total range of focus in microns for which the best theoretical resolution can be achieved, as computed from Equation (E-4). To obtain the focus tolerance d_{max} (the maximum distance from the exact focus that can provide the best resolution), the value of depth of field must be divided by two, since:

$$d_{max} = \frac{D}{2}$$

Because of the depth of field is a function of index of refraction of the immersion fluid as well as of the wavelength and N. A., for every wavelength there is a curve for dry objectives and a separate curve for each immersion fluid. For dry objectives the immersion fluid is air, whose

Table E-1

Definitions

z = resolution (in microns); the minimum distance by which two objects can be separated and still provide resolved images.

- also -

the distance in the object space corresponding to the radius of the blur circle in the image.

z_{min} = the best resolution (in microns) theoretically obtainable for a given wavelength and numerical aperture.

d = distance (in microns) from exact focus, or distance of the object from the object plane.

d_{max} = focus tolerance (in microns) or maximum distance from exact focus that can provide best theoretical resolution.

D = depth of field (in microns) the total range of focus in the object space for which the best theoretical resolution can be achieved. $D = 2\, d_{max}$.

λ = wavelength in microns.

N.A. = numerical aperture of objective and condenser.

n = index of refraction of immersion fluid for the wavelength used. For dry objectives $n = 1$ for all wavelengths.

Equations

(E-1) $$z = d \frac{\text{N.A.}}{\sqrt{n^2 - \text{N.A.}^2}}$$

(E-2) $$z_{min} = \frac{\lambda}{2\ \text{N.A.}}$$

(E-3) $$d_{max} = \frac{\lambda \sqrt{n^2 - \text{N.A.}^2}}{2\ \text{N.A.}^2}$$

(E-4) $$D = 2\, d_{max} = \frac{\lambda \sqrt{n^2 - \text{N.A.}^2}}{\text{N.A.}^2}$$

index is unity for all wavelengths; since n = 1, as the N. A. approaches 1 the value of "D" approaches zero. The greatest commercially available numerical aperture for dry objectives is about 0. 95, so values between 0. 95 and 1. 00 for dry objectives are only of academic interest. For immersion objectives, the index of refraction of the immersion fluid is also the value of N. A. for which "D" becomes zero.

The value of "D" for dry objectives is plotted for two wavelengths, 0. 546 micron in the visible and 0. 365 micron in the ultraviolet. Additional curves for dry objectives can be easily calculated from the equation.

For immersion objectives, it must be realized that as the wavelength changes the index of refraction also changes. Therefore, when calculating "D" for immersion objectives one must know the index of the liquid for each of the wavelengths to be used.

The chart shows depth of field curves for oil immersion, glycerin immersion and water immersion; most immersion objectives are designed for use with one of these liquids. Curves for both visible and ultraviolet light are given. It was assumed that the index of refraction for the immersion oil was 1. 517 at 0. 546 micron and 1. 532 at 0. 365 micron. For an objective whose specified immersion oil has indices of refraction varying significantly from these figures, new curves should be prepared from the equation. It must also be realized that the index of refraction is a function of temperature as well as wavelength, so microscopy in extreme temperatures would require different depth of field curves for immersion objectives.

3. Commercially Available Objectives

A number of commercially available microscope objectives are shown on the chart with their initial magnification plotted versus numerical aperture. Dry objectives are plotted as circles; immersion objectives are plotted as squares. Oil, water, or glycerin immersion are noted where applicable. Objectives designed for use in the ultraviolet are marked U. V.

The phrase "Long tube length" on two of the objectives signifies that these have been designed to form their primary image at a distance behind the objective which is much greater than normally used. This accounts for their higher initial magnification. All of the other objectives shown are designed for use on standard microscopes, with a mechanical tube length of about 160 mm. These are representative of commercially available objectives. It will be noted that they all lie close to a particular curve; this is caused by the relationship between initial magnification and numerical aperture which is dictated by the physical size of the objectives and the choice of tube length. If magnification greater than that shown on the chart is desired, it must be accomplished by the use of an amplifying lens or projection eyepiece, which in turn dictates a particular displacement of the final image plane from the primary image plane. (In visual microscopy, this extra magnification factor is provided by the eyepiece used.)

Table E-2 lists some commercially available U.V. objectives. The data in the table were supplied by the various manufacturers in response to a request for information; since most of the known microscope makers were contacted, the table represents most of the U.V. microscope objectives commercially available at this time. The blank spaces in the table represent information not supplied by the manufacturer.

Some of the ultraviolet objectives shown are reflecting or reflecting-refracting designs. Such designs involve the obscuration of the central portion of the aperture, which causes the resolution to be somewhat degraded. The amount of degradation of resolution is a function of the design of the particular objective.

4. Use of the Chart

Several examples will be given to illustrate how the chart can be used. Assume that it is desired to photograph (or televise) the magnified image of a specimen with a resolution of 0.5 micron at a magnification of approximately 500X in visible light. Note that the resolution curve for visible

Maker	Cat. No.	Numerical Aperture N.A.	Magnification M	Wavelength Range λ	Immers. Fluid	Focal Length F.L. mm	Tube Length T.L. mm	Working Distance W.D. mm	Quartz Slide Thick mm	Quartz Cover Thick mm	Occluded Num. Apert O.N.A	See Note
Zeiss	462060	.40	32X	.24-.70μ	Glyc.	—	160	—	.35-1.0	0-.35	0	1.
Zeiss	462063	.85	100X	.24-.70μ	Glyc.	---	160	—	.35-1.0	0-.35	0	1.
Zeiss	462064	1.25	100X	.24-.70μ	Glyc.	—	160	—	.35-1.0	0-.35	0	1.
Leitz	Q170/Q50	.50	170X	UV,VIS	Dry	2.5	~400	2.1	.5	—	.10	2.
Leitz	Q Glyc 300/0.85	.85	300X	UV,VIS	Glyc.	1.35	~400	.4	.5	—	.18	2.
C.T.S.	M8976	1.24	71X	.25-.30μ	Water	2.4	160	—	—	—	0	3.
C.T.S.	M8977	1.30	85X	.30-.40μ	Glyc.	2.0	160	—	—	—	0	3.
Band L	31-10-02	.72	53X	UV,VIS	Dry	2.8	160	.8	1.0-1.3	.18	.20	4.
B and L	31-10-03	1.0	94X	UV,VIS	Water	1.65	160	.19	1.0-1.3	.18	.16	4.
A.O.	1200	.56	50X	UV,VIS,IR	Dry	3.5	160	2.8	—	—	.28	5.

Table E-2. Some Commercially Available U.V. Objectives

General Notes:

The objectives listed can be obtained from the following companies:

Zeiss - Carl Zeiss, Inc., 485 Fifth Avenue, New York 17, New York
Leitz - E. Leitz, Inc., 458 Park Avenue South, New York 16, New York
C. T. S. - Cooke, Troughton & Simms, Inc., 91 Waite Street, Malden 48, Massachusetts
B. & L. - Bausch & Lomb, Rochester 2, New York
A. O. - American Optical, Instrument Division, Buffalow 15, New York

In addition to the objectives shown in the table, Cooke, Troughton & Simms manufacture a series of monochromats which are designed for use at a single wavelength only. These can be obtained for use at either 0.2537 microns or 0.2750 microns with the following parameters:

Focal Length	16 mm	6 mm	2 mm
Numerical Aperture	0.25	0.70	1.25
Approx. Magnification	10X	30X	85X

Condensers are available for all of the listed objectives. The Zeiss, Leitz, and C.T.S. objectives are similar in size to visual objectives; the B. & L. and A. O. objectives are much larger.

Specific Notes:

1. Refracting design. These are the objectives used in the Caspersson micro-absorption instrumentation. Quoted delivery schedule - up to 18 mos.
2. Solid reflecting design, for an object to prime image distance of 427 mm.
3. Refracting design, for a relatively narrow wavelength range. Prices to $1400.00.
4. Solid reflecting-refracting design. Two spherical reflecting surfaces plus quartz and fluorite. Not completely achromatic.
5. A completely reflecting design. Resolution quoted as 0.65 microns.

light crosses the 0.5-micron line at about N.A. = 0.55. This means that to achieve the desired resolution, the numerical aperture of the objective must be at least 0.55. However, if the greatest possible depth of field is desired, the N.A. should not be any greater than necessary. The objective shown on the chart with an N.A. of just over 0.55 is the one at N.A. = 0.6 and initial magnification m = 36X. Note that the depth of field curve for dry objectives in visible light crosses the N.A. = 0.6 line at about 1.2 micron and the resolution at N.A. = 0.6 is 0.46 micron. Since the maximum distance from exact focus that can provide best resolution is one-half the depth of field, the focus tolerance is 0.6 micron. To achieve 500X magnification with an objective of 36X, an amplifying lens or projection eyepiece must be used having a magnification of 500/36 = 14X. The parameters which the chart has helped establish are:

N.A. of objective	=	0.6
Objective magnification	=	36X
Eyepiece magnification	=	14X
Total magnification	=	500X
Resolution	=	0.46 micron
Depth of field	=	1.2 micron
Focus tolerance	=	0.6 micron

The chart can be used similarly if the wavelength desired is 0.365 micron in the ultraviolet. If some other wavelength is to be used, estimates of the curve position for that wavelength can be made, or exact values can be calculated from the equations.

Another way the chart can be used is to give information concerning the objectives plotted on it (or other objectives that can be plotted as they become known). Assume that the parameters are desired for the U.V. water immersion objective plotted at magnification = 93X and N.A. = 1.0 when used at $\lambda = 0.365\mu$. Looking down the N.A. = 1 line, it is noted that the resolution is 0.18 micron and the depth of field is 0.33 micron. The focus tolerance is therefore 0.165 micron.

A great deal of additional information could be added to the chart, but the increasing complexity might make it more difficult to obtain the most important information as now shown.

C. Immersion versus Dry Objectives

The use of immersion objectives provides two distinct advantages over the use of dry objectives: better resolution and less sensitivity to focus error. The better resolution is provided by the higher numerical apertures which are possible only with immersion objectives. The decreased sensitivity to focus error is due to the fact that because N. A. = n sin θ, an increase in the value of the index of refraction (n) allows a decrease of the angular aperture (2 θ) for the same numerical aperture. A clear example of this is seen in Figure E-1. Comparing the curves for N. A. = 0. 95 Dry and N. A. = 1. 25 Glycerin at a given wavelength, the immersion objective has better (smaller) minimum resolution (z_{min}), and a shallower slope of the curve of loss of resolution with defocusing. This is in spite of the fact that the immersion objective has a numerical aperture which is higher by a factor of about 30 percent.

The disadvantages of immersion objectives are practical ones. A layer of immersion fluid must be placed not only between the objective and object carrier but also between the condenser and the object carrier. The immersion fluid must not contain any bubbles within the light path. The design of an efficient automatic immersion fluid applicator represents an intricate problem.

The physical and optical properties of the immersion fluid must be maintained before and during use. Some immersion fluids harden when a small quantity is exposed to air over a period of time. Variation in the viscosity could cause malfunctions in an automatic dispensing system. The variation of index of refraction of liquids with temperature is much greater than for solids; therefore for best resolution, the immersion fluid should be used at or near the design temperature.

D. Visible Light versus U.V.

Ultraviolet microscopy has been developed largely because it theoretically provides better resolution than visible light as shown in Equation (E-2) of Table E-1 and because the selective U.V. absorption property of microorganisms is of great value to the biologist. In the laboratory, the principal impediment to the use of U.V. (neglecting the cost of the optics) is the need for a television system to make the image visible for observation; in the Mars Microscope, this requirement must be met in any case.

The principal consideration that indicates the desirability of U.V. for the Mars Microscope is that differential U.V. absorption seems to be the best micro method (if not the only feasible method) for providing a rapid automatic differential test for biological material.

E. Fixed Focus versus Automatic Focus

It is obvious that an automatic focusing device should not be included in the design of the Mars Microscope unless it is necessary. It is equally obvious that such a necessity arises primarily from the use of high N.A. objectives. Assuming that the more modest numerical apertures of the medium and low power objectives will permit some kind of fixed focus design, the extent of the problem with respect to the high N.A., high power objectives will be considered.

For high power objectives, the focus must be maintained to an accuracy on the order of 0.2 micron, which is about one-third of one wavelength of visible light. To achieve this with fixed focus, the object carrier should be permanently fastened to the objective by an extremely stable support, or indeed be a part of the objective. The most direct way to accomplish this is to use an immersion design and make the first element thick enough so that the front surface is the object plane. A thin specimen on the

front surface will then be in focus. Such an objective can be said to have an "integral object plane". Particles could be impacted or deposited directly on the front surface for viewing.

An "integral object plane" objective would have a number of limitations. Only the particles falling within the tiny field-of-view (20 microns diameter) would appear in the image; the "slide" could not be scanned. After analyzing the image plane, the surface would have to be wiped clean, a precise operation considering the size of the field-of-view. (It is assumed that the objective and condenser are periodically separated to allow cleaning the object surface, depositing the particles, and applying the immersion fluid which must fill the space between the condenser and the objective.)

The objective could be designed so that the object plane was below the first surface by an amount equal to the radius of the particle to be expected. For example, if the object plane was 0.5 micron below the first surface, a particle whose radius was 0.5 micron would be in focus. Assuming a focus tolerance of 0.2 micron, particles whose radius was less than 0.3 micron or greater than 0.7 micron would not be in focus. In general, this fixed focus system could be designed only for a specific particle size, where the radius varied by less than the focus tolerance.

Assume that a refracting objective is used. The integral object plane would be the front surface of the tiny hyperhemisphere, which cannot be sturdily mounted and which requires careful handling in all cases. This hyperhemisphere magnifies the object by a factor equal to its index of refraction, or about 1.5 times; the remaining magnification is supplied by the succeeding elements. Therefore, not only must the tiny front element be of exact thickness, but it must remain in exact position with respect to the other elements. A rigorous treatment of this point is not possible here, but as an indication of magnitudes it can be stated that for the usual refracting objective design, the temperature would have to be kept within about 3°C of design temperature. Vibration or shock might make this fixed focus system completely inoperative.

With a reflecting objective, vibration and shock are not nearly as serious a problem, but the temperature must still be maintained within limits. Assuming a two mirror solid design objective of fused quartz, it has been estimated that the temperature would have to be within 6°C of its design value. Also, as will be discussed later, the reflecting objectives cannot be obtained with the highest numerical apertures.

These comments indicate the problem of establishing a fixed focus system for high numerical apertures. The values here are rough estimates; a thorough analysis of particular objectives would be necessary to provide exact data. However, the intent of these comments is to establish the fact that if an automatic focusing system is not provided, the problems suggested here must be thoroughly investigated and the solutions to these problems must be incorporated into the final design.

F. Temperature Effects

The effect of temperature on a fixed focus system and on the immersion fluid index has been mentioned. In addition, at temperatures much different from room temperature the separation, thickness, radii of curvature, and index of refraction of the lens or mirror elements will be changed. The extent to which the resolution will be degraded thereby is a complex function, which should be investigated for the particular objective design if highest resolution is required for widely varying temperatures.

G. Reflecting versus Refracting Objectives

The three basic configurations of microscope objectives are: 1) the refracting objective, in which as many as ten small lenses are mounted in individual cells which nest in a precise cylinder, 2) the reflecting objective, in which two mirrors are mounted in the form of an inverted Cassegrain telescope, sometimes with lenses in the hole in the larger mirror, and 3) the solid reflecting objective, which is essentially a two-mirror design but which mechanically is a single piece of quartz.

The solid reflecting objective represents the design least sensitive to vibration, shock, and atmospheric conditions, although as discussed previously, none of these designs will maintain fixed focus over a large temperature range. One disadvantage of reflecting objectives is the degradation of resolution because of the obscured portion of the aperture and the possibility of stray light. For high N. A. objectives, these effects can be minimized but not nullified. Another disadvantage of reflecting designs is the limitation of numerical aperture imposed by the limited angular aperture (2θ) inherent in mirror objectives. In Table E-2, for example, the highest N. A. for a reflecting objective is 1. 0 for water immersion, which implies an angular aperture of 96 degrees; the highest N. A. for a refracting objective is 1. 3 for glycerin immersion, which implies an angular aperture of 122 degrees.

A factor to be considered when selecting U. V. objectives is that some of the designs use lenses of calcite (calcium carbonate), fluorite (calcium fluoride), or lithium fluoride. These materials are more sensitive to certain environmental conditions than fused quartz and should be handled accordingly.

H. Proper Focus versus N. A.

It is restated here for emphasis that highest numerical aperture is desirable only when the focus is kept within the depth of field; otherwise it is better to use lower N. A. This can be seen in reference to Figure E-4, for example. The best (smallest) resolution is provided by the N. A. = 1. 4 objective for perfect focus; however, if the focus is in error by as little as $d = 0.1\mu$, the resolution of the N. A. = 1. 4 objective is degraded from 0. 13 micron to 0. 27 micron, while the N. A. = 1. 0 objective is still 0. 18 micron, and in fact remains so until about $d = 0.2\mu$. This means that the focus accuracy should be determined first of all, and the N. A. chosen accordingly. If the highest N. A. is required to provide the highest resolution, the focus must be kept within the depth of field.

I. Problems in New Designs

As mentioned previously, the ten objectives listed in Table E-2 represent most of the U. V. objectives commercially available at this time. If the Mars Microscope requires objectives whose parameters are different from any of those listed, it will be necessary to design new objectives, just as it will be necessary to design a special amplifying lens to minimize the overall length of the system. A few comments regarding the extent to which a redesign can be successful are in order here.

The maximum N. A. which can be expected from a reflecting objective is about 1. 1 for glycerin immersion (n = 1. 49 for U. V. λ = 0. 365μ), although higher N. A. could be achieved if a suitable immersion fluid having a higher index of refraction and adequate U. V. transmission can be found.

The degree of aberration correction which can be achieved in a reflecting objective will vary inversely with N. A. for the higher values of N. A. It should be remembered that the nominal N. A. of the objective determines resolution only when the aberrations are adequately corrected. Refracting objectives, even at high N. A., can usually be corrected adequately; reflecting designs present greater problems in this regard.

The desirable use of materials possessing low coefficients of thermal expansion for the lens cells in the refracting objectives represents much difficulty in fabrication. If large variations in temperature are to be experienced, the cements used for both refracting and reflecting designs must be investigated.

The fabrication techniques used in the manufacture of microscope objectives are extremely delicate. New designs which demand new techniques cannot be achieved hastily. Therefore, it is of utmost importance that the requirements for new designs be established and the actual design work initiated in the Mars Microscope program as soon as possible. This applies not only to the objectives, but also the condensers and amplifying lenses.

J. Light Sources and Detectors

If a vidicon is used as the detector in the Mars Microscope optical system, the important parameters for the light source are:

1) Light output in the desired spectral region

2) Power consumption

3) Shape and size of the radiant area

4) Size and weight of the lamp and associated equipment

If visible light is used, a tiny tungsten filament lamp would provide sufficient energy with minimum power requirement. The filament configuration should be chosen to provide the most efficient use of the light and also to provide the best resolving power for video-micrography.

If U. V. is used, the choice of the light source becomes a fundamental problem. The U. V. sources in current use are primarily mercury arcs, xenon arcs, and hydrogen discharge sources. These are not easily miniaturized and their power requirements far exceed that of the tiny tungsten filament lamp. The problem becomes more acute when narrow band pass filters are used to provide nearly monochromatic radiation for differential absorption measurements; in that case, only a very small part of the source output is used. Intermittent exposures would help to minimize the power requirement, but it raises another difficulty. It was noted* that vidicon response fell to 10 percent of normal after a few U. V. flash exposures with 17 seconds spacing. (Illumination of the vidicon faceplate with white light between exposures was suggested as a means to avoid this effect, but this introduces another power requirement and its efficacy is not completely determined.)

* Williams, G. Z. Time lapse ultraviolet microscopy instrumentation and biological applications. IRE Trans. on Medical Electronics, ME-6, 2, 68, June 1959.

It would be possible to use a flying spot scanner and a photocell to replace the vidicon in the optical system, as described elsewhere in this report. This does not solve the power requirement problem, however, regardless of whether the source is a cathode ray tube or an illuminated pinhole.

The task of optimizing the light source and detector must be considered in relation to the overall problem of observation and detection. An experimental program is required to determine first of all the best means for automatic biological observation and detection using the various sources and detectors in their existing configurations. Only then will it be possible to choose intelligently the most feasible approach to miniaturization of these components.

K. Percentage of Field Covered by Particles

The basic functions of the Mars Microscope optical system are detection, analysis, and video-micrography of biological particles. In considering the optimum percentage of the field to be covered with particles, it will be recognozed that although the probability of detection increases with the number of particles, an upper limit is reached when the individual particles lose their identity. The same upper limit applies for detection by illuminance differences in the focal plane, neglecting detection by change in transmission of the total field-of-view. Therefore, for detection and video-micrography, the optimum arrangement would be a single layer of particles separated only by enough distance to maintain their individual identity. This assumes that the layer of particles is at or near the fixed focus object plane or one of the preselected object planes for automatic focus.

For detection or analysis by selective U. V. absorption, it might seem that the optimum arrangement would be a closely packed clump of particles; this would be true if the particles were composed of a single substance or

several substances with identical absorption and refraction characteristics. This is not generally the case, and the best absorption measurement techniques usually involve isolating individual particles, and even selected portions of individual particles. Therefore, it appears that detection, analysis, and video-micrography could all be best accomplished on a single layer of particles separated only by enough distance to maintain their individual identity.

L. Cleaning an Integral Object Plane Objective

In considering the use of an objective whose initial surface is the object plane on which particles can be deposited for immediate fixed focus viewing, the problem of cleaning the surface arises. For direct impaction on an immersion objective, it must be remembered that immersion fluid must be applied before viewing. (Whether one can apply the fluid without either leaving air bubbles in the optical path or dislodging the impacted particles remains a problem for further study.) To remove the particles, it would appear feasible to first use a small "brush" impregnated with immersion fluid and then another "brush" with a suitable solvent. If it is feasible to impact on a very thin film of immersion fluid, it might not be necessary to use the solvent. Obviously, the design of "brushes" to thoroughly clean an area as small as 20-micron diameter is no simple task.

The cleaning problem is obviated if electrohydrodynamics (E. H. D.) is used for concentration of the particles on the integral object plane. The great majority of the particles are easily removed by reversing the polarity of the electrodes and flushing the liquid through the chamber.

It should be noted that a few particles remaining in the field-of-view will not impair subsequent operation of the microscope, as long as the percentage of their field coverage is small. Much more serious is the possibility of a "hair" from a cleaning "brush" being left in a position to block the field-of-view entirely.

M. Preselected Object Planes

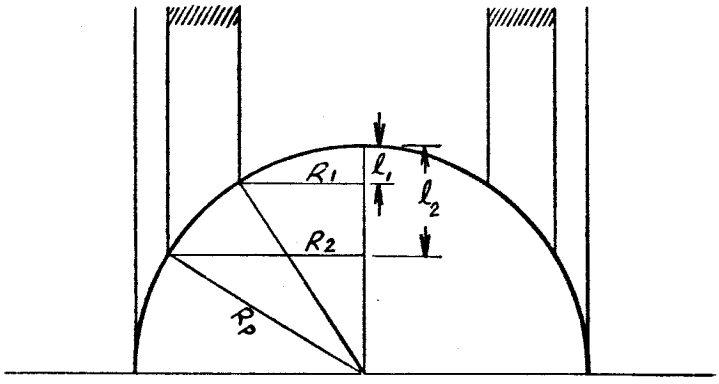
One of the objectives being considered for the high magnification of the Mars Microscope is the Zeiss 100X, glycerine immersion, N.A. = 1.25, with an optimum resolution of 0.15 microns over a depth of focus of 0.2 microns (when used in the U.V. wavelengh of 0.365 microns). In order to illustrate how this high-power objective might be used with automatic focus at preselected object planes, consider the use of three such planes. Assume that the objective will be focused at 0.15, 1.0, and 2.0 microns from the slide surface for each field of observation, and that the field contains particles in the size range of 0.5 to 10 microns diameter. This combination of three focus positions provides a good compromise in overall resolution for bacterial size particles (on the basis of the curve labeled "N.A. = 1.25" in Figure E-4, which shows the degradation of resolution as a function of increasing distance from the exact focus position.

Our choice of focus plane positions represents a compromise based upon a consideration of resolution values at the top and along the equator of different size particles. However, the question arises as to how the resolution of individual elements along the particle surface varies within the vertically projected plane image of the particle. For example, if the plane of exact focus is at the top of a spherical particle, how does the resolution vary within the projected image from the top to the equator of the particle? If certain optical and biological subtleties are momentarily neglected, the problem may be studied with a simple geometrical model, if it is assumed that the particles are spherical, and if we limit our concern to the resolution of the particle surface itself and momentarily ignore the more complex question of resolving structural details within the particle.

Since we can compute from optical theory the value of resolution as a function of distance from the plane of exact focus, we can also compute the resolution corresponding to each latitude circle along the particle surface from its pole to equator. If we then consider a latitude zone where the

resolution has a defined range of values, we can calculate the percentage of the total particle area over which this range of resolution applies by computing the area of the annular ring vertically projected from the two latitude circles.

The following diagram shows the geometrical considerations which apply to our problem:



From the particle geometry,

$$R_p^2 = R_2^2 + (R_p - \ell_2)^2, \quad \text{or} \quad R_2^2 = 2R_p\ell_2 - \ell_2^2$$

$$R_p^2 = R_1^2 + (R_p - \ell_1)^2, \quad \text{or} \quad R_1^2 = 2R_p\ell_1 - \ell_1^2$$

$$A_R = \text{Area of projected annular ring} = \pi(R_2^2 - R_1^2)$$

$$A_T = \text{Total projected area} = \pi R_p^2$$

$$\frac{A_R}{A_T} = \frac{\pi (R_2^2 - R_1^2)}{\pi R_p^2} = \frac{(2 R_p \ell_2 - \ell_2^2)}{R_p^2} - \frac{(2 R_p \ell_1 - \ell_1^2)}{R_p^2}$$

Values of A_R/A_T were computed from a graph which expressed the relative area of projected circles centered at the particle pole in terms of the ratio ℓ/R_p. The nature of this graphical relationship is shown by the following:

ℓ/R_p	Relative Area (%)
0.1	19
0.2	36
0.3	51
0.4	64
0.5	75
0.6	84
0.7	91
0.8	96
0.9	99

By means of this method, preliminary calculations were made for two sample cases where the center of focus was placed at 0.5 and 1.0 microns above the object plane surface. Tables E-3 and E-4, following, present for a suitable range of particle diameters, the calculated values of the observable percentage of vertically projected particle surface area with at least the given values of resolution. It is obvious from these results that our simple scheme for stepping the objective to these fixed positions will produce a serious "hole" in the resolution quality for 0.9-micron diameter particles. Also of interest is the result that no particle under these circumstances can be completely resolved to a value of 0.2 microns. On the

Table E-3. Observable Percentage of Vertically Projected Particle Surface Area with at least the Given Value of Resolution

(Zeiss 100X U.V. - glycerine immersion objective; optimum resolution 0.15 microns; depth of focus 0.2 microns; center of focus 0.5 microns above object plane surface)

	Resolution (μ)												
Particle Diameter (μ)	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.2	1.4	1.6	1.8	2.0
0.3	--	0												
0.4	32	74	96	(100)										
0.5	79	88	96	100										
0.6	95	100	100	100	100									
0.7	66	98	100	100	100	100								
0.8	34	55	82	100	100	100	100							
0.9	17	31	48	70	96	100	100	100						
1.0	7	16	28	42	61	83	100	100	100					
1.2	1	3	7	14	24	35	49	65	84	100				
1.4	--	--	1	3	7	13	21	30	41	68	100			
1.6	--	--	--	--	1	4	7	12	19	36	57	84	(100)	
1.8														
2.0														

Table E-4. Observable Percentage of Vertically Projected Particle Surface Area with at least the Given Value of Resolution

(Zeiss 100X U.V. - glycerine immersion objective; optimum resolution 0.15 microns; depth of focus 0.2 microns; center of focus 1.0 microns above object plane surface)

	Resolution (μ)															
Particle Diameter (μ)	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.2	1.4	1.6	1.8	2.0	2.2	2.4	2.6
0.6	--	--	--	--	--												
0.7	--	--	--	14	45	69	(86)	(96)	(100)								
0.8	--	--	28	53	72	87	96	(100)									
0.9	15	40	60	76	88	96	99	100									
1.0	47	64	78	88	95	99	100	100	100								
1.2	61	88	95	99	100	100	100	100	100	100							
1.4	33	49	65	80	97	100	100	100	100	100	100						
1.6	17	25	34	43	55	67	81	96	100	100	100	100					
1.8	7	11	16	23	30	38	47	58	69	95	100	100	100				
2.0	2	4	7	11	15	21	27	34	42	60	82	100	100	100			
2.2	--	1	2	4	7	10	14	19	25	37	53	72	94	100	100		
2.4	--	--	--	1	3	5	7	10	14	23	35	48	65	83	100	100	
2.6	--	--	--	--	1	2	3	5	7	13	22	32	44	58	74	93	100

basis of our geometrical model, it is only in the case of very small particles, e.g., 0.2 to 0.4 micron diameter, when the center of focus is some 0.3 to 0.4 microns above the object plane surface, that the entire hemispheric surface can be resolvable to such a value.

On the basis of these preliminary considerations, it would appear that the use of only a few preselected object planes for the high-power microscope automatic focus device can only be justified if we are willing to accept periodic "holes" in the fine resolution quality. The results suggest, therefore, that we might have to consider infinite tuning of an auto-focus device using a photo-detector to individually optimize the image quality of particles within the field-of-view. As explained elsewhere in this report, such an alternative presents numerous difficulties.

III. AUXILIARY INSTRUMENTATION

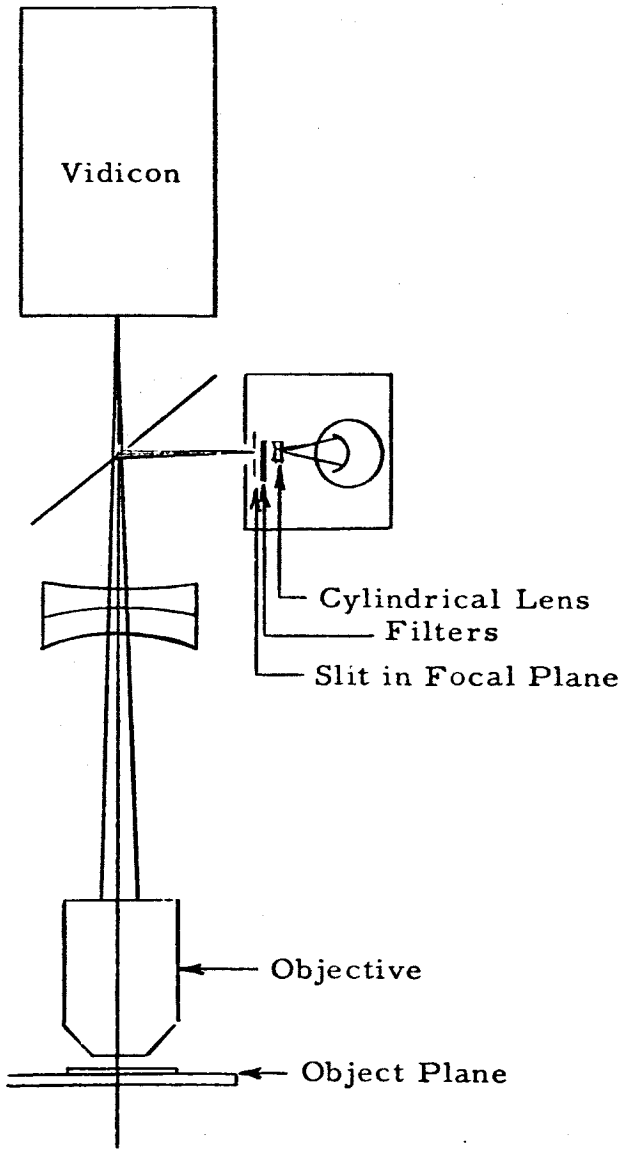
A. Particle Detection and Centering

One goal of the Mars Microscope design is to provide video micrographs not of individual particles whose biological nature have been positively determined, but of a field-of-view in which are concentrated the majority of particles possessing significant characteristics that have been isolated from a sample of the planetary atmosphere or surface. In particular, it is suggested that particles of a given size and density provide a high probability of being biological. Subsequent analysis on earth of the videomicrographs of these particles might provide more positive proof based on recognition of the particle form or shape. A system based on this concept would not require single particle detection. (Automatic focusing can be accomplished without such detection as explained elsewhere in this report.) However, if the optical system is required to center individual particles in the field-of-view for spectrophotometric analysis, for example, an automatic particle detection function is necessary.

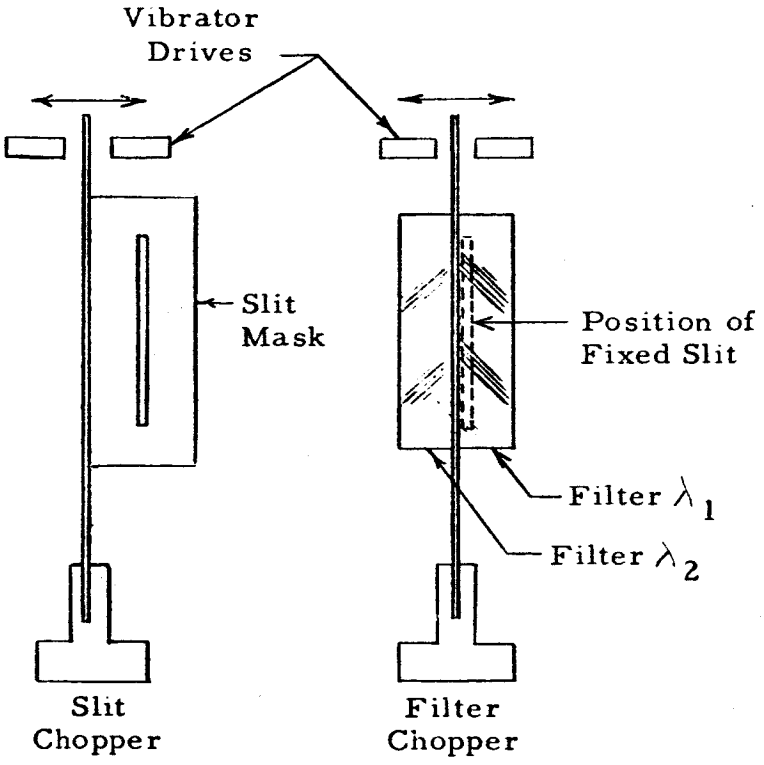
Automatic particle detections can be accomplished by measuring illuminance differences in the image plane. For example, the signal from the vidicon could be analyzed to locate a dark spot on the tube face caused by a particle in the field-of-view, assuming the particle has some absorption. The resultant information could be used with appropriate logic circuitry and control mechanisms to bring the particle to the center of the field-of-view. An alternative particle detector might use two vibrating slit choppers of the type shown in Figure E-6 in front of a photocell to analyze the illuminance pattern in the image plane. The choice of one of these methods or the use of other possible image scanners depends largely upon the complexity, size, weight, and power requirements of the necessary electronics and mechanical devices. It should be stressed that the logic required to center one particle when many particles are in the field-of-view becomes formidable.

B. Actuators and Bearings

Some of the motion functions associated with the optics are objective focus, sample slide drive and scan, objective changing and image chopping. If the sample can be concentrated into a very small area, the range of motion necessary for the sample scan as well as for the objective focus would be very small. These motions could be accomplished by the use of "frictionless bearings" which are essentially systems using parallel leaf spring supports. One such bearing is shown schematically as the focus adjustment mechanism in Figure 2, in the main body of this report. As shown in the figure, the actuator could be electromagnetic. Alternative actuators could be piezoelectric, or could involve stepping motors with differential-screw type drives. It would be possible to accomplish the sample scan and the objective focus by moving only the objective as shown in the figure; it would also be possible to eliminate the dovetail bearings and use the spring-support frictionless bearings for all three directions of objective motion.



Simple Spectrophotometer
on Microscope (Schematic)



Vibratory Drives for Image
Scanning and Spectral Analysis
(Schematic)

Figure E-6

If it is desired to use multiple objectives on a microscope tube, the objectives could be mounted on the usual type of rotating multiple nosepiece. The movable part of the nosepiece could incorporate a gear on its periphery which would be driven by a small motor. Mechanical detents would establish the exact position of the nosepiece when the objective is centered in place. A small d-c motor with gearhead to drive the multiple nosepiece might weigh about 4 ounces and consume about 3 watts of power. The disadvantage of a d-c motor is its brushes, which can pose a reliability problem. An a-c motor could be used, but the efficiency of small two-phase a-c motors is low (about 10 percent compared to 50 percent for a d-c motor). Brushless d-c motors may be available before too long. Kearfott is now working on a brushless d-c motor which uses Hall effect generators for commutation*. An alternative drive would use a solenoid and ratchet to drive the nosepiece. A typical solenoid which might be used would weigh about 3.5 ounces and consume 20 watts to give a force of 20 ounces with a 1/8-inch stroke.

Image chopping (or mechanical scanning of the image for photometric differences) is discussed elsewhere under particle detection. It should be noted, however, that vibratory chopper drives such as those illustrated in Figure E-6 are efficient and reliable. Numerous motor-driven chopper designs are also available.

An energy source for the mechanical drives that might be considered is the coiled spring. A tiny solenoid could trigger an escapement which would release the energy in discrete pulses to ratchet type drives.

C. A Simple Spectrophotometer

The existence of ultraviolet absorption bands in the transmission spectra of biological matter provides one simple method for differentiating between biological and non-biological matter. Complex transmission curves

*Military Systems Design, Nov.-Dec. 1961, p. 14

can be used to definitely identify certain substances and to measure their concentrations. For the Mars Microscope, a complex scanning micro-spectrophotometer is not feasible. However, significant data can be derived from a relatively simple system. One can measure the relative transmission through two broadband pass ultraviolet filters, one in the wavelength region of high transmission and one in the region of high absorption.

Assume that part of the energy directed to the focal plane of a microscope is focused on the fixed slit of a "simple spectrophotometer" as illustrated in Figure E-6. Behind the slit is a vibrating reed on which are mounted two tiny pieces of quartz "cover glasses" on which spectral band-pass filters have been deposited. Behind the "filter chopper" is a cylindrical lens which spreads the beam to the surface of a photocell. The filter chopper is energized so that the radiation passes through each of the two filters sequentially. Assume that the output of the photocell has been set so that with no specimen in the microscope field-of-view, the signal has the same intensity for both filters. If a non-biological specimen is placed on the microscope stage, in general the material will absorb some of the energy but will absorb the same percentage of energy in both wavelength regions, and although both phases of the photocell signal will be reduced, their ratio will still be unity. If a biological specimen is placed on the microscope stage, it will absorb more strongly in one wavelengh region than the other, and the ratio of the two phases of the photocell signal will be different from unity. This result can be used to make the differential analysis. (Even if the calibrating signal ratio is not unity, the difference in the ratio will provide the same information.)

If the specimen image occupies only a portion of the slit, the change in signal ratio from unity will be less pronounced. The width and length of the slit must be chosen with this in mind, while also considering the utilization of the maximum energy possible and the possible methods of scanning the slide for objects.

The vibratory filter chopper is highly desirable because of its simplicity and low power requirements. The vibratory slit chopper which is also shown in Figure E-6 might be used for photometric measurements as described in this report under "Particle Detection".

D. Blink Comparator

One method of detecting biological material by its differential absorption in the ultraviolet spectrum would utilize a technique used in astronomy. If two pictures of the same stellar region, but taken at different times, are viewed sequentially in a "blink comparator", any change in the position or brightness of a single star is readily detected, even when there are thousands of non-varying stars in the picture. This occurs because the eye is more sensitive to differences than to fixed objects. If two photomicrographs taken at different wavelengths are viewed sequentially, and if parts of the pictures varied in brightness, this fact would also be detectable. If the pictures consisted of a large number of small particles only a few of which were biological, the few would exhibit the blink characteristics.

This method is suggested if it is not found feasible to detect the differential absorption of single bacteria by automatic means contained in the microscope. The only spaceborne components required are the two filters.

For this method, it might be desirable to provide recognizable particles with no differential absorption on the slide so that a "calibration" would be included.

E. The Flying Spot Scanner

In a flying spot scanner, the light source may be an illuminated spot on the face of a cathode ray tube or an illuminated pinhole. Reversing the direction of light from that in the usual microscope system, the source, which is in the final "image plane", is imaged in the object plane by the objective. The image of the source is scanned across the object by the

scan of the cathode ray tube, or by an oscillating mirror system when the fixed light source is used. The energy transmitted by the object falls on a photocell; no condenser is necessary beneath the object in the classical sense, although a lens system may be used to bring the energy to the photocell in the optimum fashion. For display purposes, the output of the photocell can be fed to a second cathode ray tube, whose sweep is synchronized with the light source scan. For transmission, the amplified signal of the photocell can be used directly.

One virtue of the flying spot scanner is that the object is irradiated by the illuminating energy only periodically. This is of special importance when U. V. is used, because of the harmful effect of prolonged U. V. irradiation on organisms.

In considering the resolution achievable with a flying spot scanner, the following definitions are applicable:

r = radius of light source (in microns)

r' = geometrical radius of light source image (in microns)

m = magnification of objective when used alone, or magnification of objective-amplifier combination

z_{min} = best theoretical resolution of an object illuminated by a condenser (in microns)

z_o = best theoretical resolution of a self-luminous object (in microns)

λ = wavelength (in microns)

N. A. = numerical aperture of objective

In order to determine the resolution achievable with the flying spot scanner, one must first compute r' and z_o according to the equations

$$r' = \frac{r}{m}$$

$$z_o = 2\, z_{min} = \frac{\lambda}{N.A.} \qquad \text{(from diffraction theory).}$$

When diffraction is neglected, r' is the radius of the image of the light source on the specimen, and therefore, would also be the resolution. However, because diffraction ultimately limits the image radius, the resolution can be no smaller than z_o. Therefore, r' is the resolution when r' is greater than z_o, and z_o is the resolution when r' is smaller than z_o. In other words, the resolution of a flying spot scanner is the larger of the quantities r/m and λ/N.A. (Because of the interaction between the geometrical and diffraction effects, this is not an exact relationship, but it is sufficiently accurate for most purposes.)

Note that the best theoretical resolution achievable with a flying spot scanner is worse (larger) by a factor of two than that achievable with the same objective when used with a condenser in conventional microscopy.

To calculate the largest light source that can be used and still achieve the best theoretical resolution with the flying spot scanner, let

$$r = m\, z_o = 2\, m\, z_{min} .$$

For example, if m = 1000X and $z_{min} = 0.2\mu$, then r = 400μ. The spot on the phosphor or the illuminated pinhole should, therefore, be no larger than 0.8 mm in diameter. (In practice, the diameter would be made somewhat smaller than 0.8 mm, because of the interaction of geometrical and diffraction effects.)

F. Two Automatic Focusing Devices

Two devices which might be used for maintaining the focus of the Mars Microscope are discussed here in enough detail to provide a basic understanding of their design principles and limitations. The device designed

and built by the Research Division of the Polaroid Corp. has been operated successfully for some years. The device designed and fabricated by Log Etronics Inc. has been used for semi-automatic focusing of copying cameras and enlargers.

1. The Polaroid Automatic Focusing Device*

The principle of the Polaroid automatic focusing device can be best explained with reference to Figure E-7. The "Simple System" is a schematic of the focusing device in its minimal form, the dashed lines enclosing the components which must be added to the usual microscope setup. Light from a source lamp goes through a beam splitter, a field lens, a pinhole, and the microscope objective, the objective focusing a minified image of the pinhole in the object plane. At a glass surface such as the top of a microscope slide, most of the energy will be transmitted, but approximately 4 percent will be reflected back toward the objective. If the top of the slide is precisely in the object plane, the reflected light will be magnified by the objective, re-imaged precisely on the pinhole, will pass through the pinhole, will be partially reflected by the beam splitter and will fall on the phototube which will produce a signal on the meter. If the top of the slide is below or above the object plane, the reflected light image of the pinhole is not in focus at the plane of the pinhole. Because a blur circle is formed at the pinhole, only a portion of the light is transmitted through the pinhole, and a smaller meter reading results. This simple proof is adequate to show that a peak meter reading results when the top of the slide is in precisely the object plane.

If a vidicon tube is placed with its image surface coplanar with the pinhole, then a thin specimen lying on the surface of the slide will be in perfect focus on the vidicon tube when the peak meter reading is achieved.

* Baxter, L. et al. New principle for focusing a high power microscope and means for accomplishing the focusing automatically and with great accuracy. J.O.S.A., 47 (1): 76 (Jan. 1957).

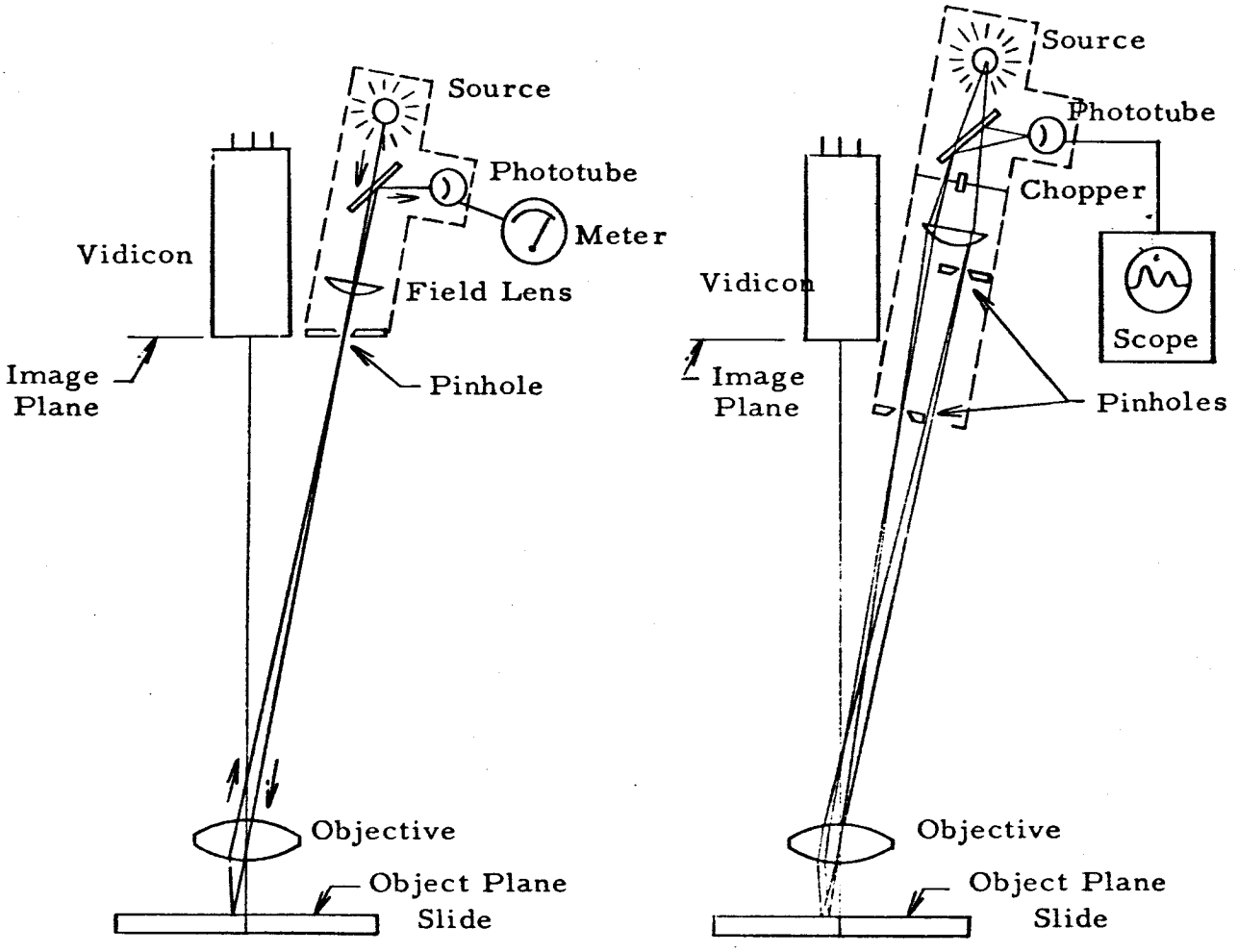


Figure E-7. Automatic Focusing Device, Polaroid Corp.

It should be noted that in theory none of the light from the focusing device gets into the vidicon, although in practice an inconsequential amount of scattered light may fall upon the vidicon.

The fully automatic device shown in Figure E-7 utilizes two pinholes and a chopper in addition to the components found in the simple system. This arrangement provides essentially two simple systems, whose peaks result when the top of the slide is somewhat below focus for one pinhole and somewhat above focus for the other pinhole. This provides direction information for the focusing servo, the system being calibrated to be in focus when the two signals are equal. A slightly unequal condition, and therefore a slightly out of focus situation, is shown by the waveform on the oscilloscope in the figure.

It should be noted that the top of the slide can be kept in focus even when there is no specimen in the field-of-view. It should also be noted that tilting the slide a small amount will not introduce appreciable error because even when the slide is tilted the rays from the pinhole image in the object plane are still re-imaged on the pinhole, as long as the pinhole image is in focus on the slide. Pronounced tilting of the slide could cause second order effect errors, but that can be prevented with little difficulty.

The automatic focusing device can maintain focus within about 0.1 micron for extended periods of time, even while the slide is in motion. The equipment operates irrespective of changes in index of immersion fluid, flatness of the slide, and temperature effects in the various optical and mechanical components.

In the above discussion it was assumed that the specimen was thin and lying directly on the top surface of the slide. If an immersion objective is used, the index of the immersion fluid must be sufficiently different from the index of the slide to retain an adequately reflecting interface at the object plane. Alternatively, the top of the slide can be coated with a partially reflecting film. The same considerations hold for a specimen under a cover glass in a mounting medium.

Because the focusing principle utilizes the reflecting surface of the top of the slide, it is necessary that no other reflecting surface be allowed to confuse the device, causing it to "lock on" to the wrong surface. This might require limiting the focusing motion to prevent focusing on the bottom of the slide. If a cover glass is used, the focus could be approached always from below, to prevent focusing on the top of the slide.

In order to discuss additional important features of the automatic focusing device, it is necessary to realize the relationship of object distance and image distance in microscopy. The formula of interest is:

$$\Delta \ell = \frac{\Delta \ell'}{m^2}$$

where

$\Delta \ell$ = change in object distance

$\Delta \ell'$ = change in image distance

m = magnification

For reasons that will be apparent later, let us choose $\Delta \ell' = 50$ mm. Also, assume that the magnification shown are initial magnifications, which can be increased by a factor of 10 in a subsequent imaging system. Some data of interest are:

$\Delta \ell'$	m	$\Delta \ell$
50 mm	100X	5 microns
50	50X	20
50	10X	500

We have thus far assumed that the specimen is "thin". Because we are looking for particles that have thickness, it might be desirable to focus on a plane somewhat above the slide. Considering the "simple system" this could be accomplished by displacing the pinhole down from the image plane by an amount $\Delta \ell'$, whereupon the microscope would be in focus for a plane which is $\Delta \ell$ above the top of the slide. A reasonable distance to move the pinhole in the standard microscope configuration is about 2 inches, or 50 mm. The table shows that this will allow focusing at a distance above the slide of 5 microns at 100X, 20 microns at 50X, and 500 microns at 10X. These figures establish reasonable amounts of "focus bias" for the initial magnifications given. For example, if we were expecting 8-micron diameter particles, we might want to focus 4 microns above the slide at high magnification. It is also possible to bias the focusing electrically, but within essentially the same limits as for the method described.

Returning to the simple system without any "focus bias", consider the use of a cover glass and a mounting medium, assuming that we were focusing on the reflecting surface of the top of the slide. The cover glass "optical thickness" is usually about 100 microns, so there will be an additional reflecting surface (the top of the cover glass) which will reflect light toward the pinhole. Without trying to prove the point rigorously, it can be stated that at the higher powers, the top of the cover glass will be sufficiently out of focus to cause any ambiguity, but at lower powers the false signal will begin to cause errors. This factor must be considered in the final design.

The Polaroid automatic focusing system as it now exists can meet the most severe requirements for a particular microscope-specimen combination. However, use of several magnifications, both dry and immersed objectives, different specimen thicknesses, and specimen mounting in a medium under a cover glass or on the bare top surface of the slide will demand a careful redesign to insure compatibility with all these different parameters.

2. The Log Etronics Focusing Meter*

The Log Etronics Inc. focusing meter makes use of the non-linear response of a photodetector to provide a peak signal voltage when an image is in best focus on the detector. Operation of the instrument, with photocells whose response is approximately logarithmic, depends upon the fact that log (A + B) is not equal to log A plus log B. A pair of photocells is used, one of which is prevented from receiving a sharp image by use of a diffusing material, and their differential output is measured in a bridge circuit.

The operation of the instrument can be explained without the use of equations by the following comments. As a unit area of the photocell is flooded with increasing light, the signal increases at a rate greater than the light level. If an image is projected in sharp focus on the photocell, (such as a grid with half the pattern bright and half the pattern dark), a voltage signal will result. If the image is then thrown out of focus in such a way that all the light still falls on the photocell but is distributed uniformly over the surface of the photocell, the voltage signal drops. This occurs because in the focused condition all of the light is incident at a given level of illuminance, whereas in the out-of-focus condition all of the light is incident at one-half the original illuminance. The cell output per unit of incident light flux is greater for higher illuminance levels (logarithmic response). Therefore, the cell output will be greatest when the maximum light flux falls on the minimum area of the photocell. (A simple mathematical proof is included later in this report.)

It would be possible to obtain an indication of focus by noticing the peak signal as the focus was varied. In practice it is more feasible to get the peak of voltage difference between the cell and another cell that always

*Craig, D. Photoelectric measurements of optical focus. J.O.S.A., 51 (12): 1456 (Dec. 1961).

receives an out-of-focus image. The "active" cell is placed in the desired focal plane, and the reference cell is placed nearby, with a diffusing screen covering it. When an out-of-focus image is projected on the two cells, the cell outputs are balanced in a bridge circuit to give no signal. As the image is brought into focus on the "active" cell, its output voltage increases, while the reference cell output remains the same, since it is always "out-of-focus". Therefore, the voltage difference of the two cells increases, until it is peaked at the condition of best focus.

The Log Etronics focusing meter can automatically detect the in-focus condition, as demonstrated at the Optical Society Meetings in October, 1961. However, there are several problems that would be encountered in considering its use for the Mars Microscope. The meter can detect the in-focus condition of a fine, high contrast, regular shaped pattern positioned in one definite plane. It would not operate well with a normal biological specimen image whose structure would be of moderate to low contrast, both fine and coarse in texture, and which would appear partially in focus in several different planes. One solution to these problems would be to provide a test slide carrying the necessary grid pattern to calibrate the focus and then to maintain the same object plane for the specimen slides. This could be done, but it is not an altogether appealing method. It is possible, however, that some of the Log Etronics focusing meter features can be incorporated into a device which uses other principles as well.

3. Simple Mathematical Proof of the Log Etronics Focusing Principle

Assume that a focused pattern of light falls on a detector, with some bright areas and some dark areas.

A_3 = area of detector

A_1 = bright area

$A_3 = CA_1$

A_2 = dark area

E = illuminance in $\frac{\text{flux}}{\text{area}}$

F = light flux = $E \times A$

The total flux would be

$$F = E_1A_1 + E_2A_2 \quad .$$

If no flux falls in the dark areas,

$$E_2 = 0 \text{ and } F = E_1A_1 \quad .$$

Now, if all the energy is somehow diffused to cover uniformly the entire area of the detector, as in the out-of-focus condition,

$$F' = E_3A_3 \quad .$$

Since

$$F = F', \qquad E_1A_1 = E_3A_3 \quad .$$

If the ratio of the total area to bright area was originally

$$A_3 = CA_1$$

then

$$E_1 = CE_3 \quad .$$

Consider a non-linear detector whose output voltage per unit area is not proportional to the illuminance. Now,

$$E_1 = CE_3, \quad \text{but} \quad \frac{V_1}{a} \neq \frac{CV_3}{a}$$

So in general, let

$$\frac{V_1}{a} = kC \frac{V_3}{a} ,$$

where k is the non-linearity factor of the cell for the two illuminance levels.

The total voltage for the flux

$$F = E_1A_1 \text{ is } V = \frac{V_1}{a} A_1$$

or

$$V = kc \frac{V_3}{a} A_1$$

The total voltage for the flux

$$F' = E_3A_3 \text{ is } V' = \frac{V_3}{a} A_3$$

or

$$V' = \frac{V_3}{a} CA_1$$

since $A_3 = CA_1$. Therefore

$$\frac{V}{V'} = k .$$

So the voltage output of the cell is higher for the in-focus condition by a factor of k, which is the "non-linear factor" assumed for the detector. The simple proof given here establishes that the output of a non-linear photodetector is peaked when the image falling upon it is in sharpest focus.

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APPENDIX F

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1. Can bacteria be separated by density? Do they not cling to denser materials?

2. p. 12 — biologist needed?

3. p 18 - recommended steps