

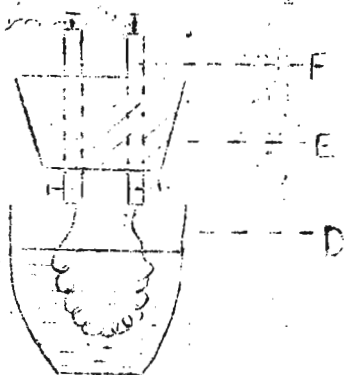
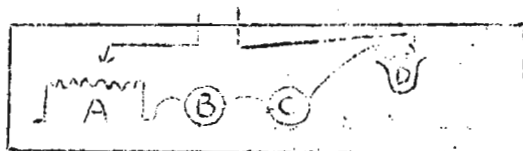
Kaufmann, B. P. Technique for spreading salivary chromosomes.

more uniform distribution of cells and less breakage by flattening with a weighted roller rather than with a needle or similar instrument, where the results depend on the pressure exerted by the technician. For this purpose we use pieces of glass tubing, filled with about 150-200 grams of mercury, and securely corked. A horseshoe-shaped wire with the ends inserted in the corks may be used as a handle in drawing the roller across the cover.

Griffen, A. B. Sealing slides with paraffin.

In the preparation of salivary chromosome slides for rapid checking of breaks and translocations, it is seldom desirable to spend long hours in making permanent mounts of all the preparations; it is far more practicable to make well-sealed temporary slides which may be made into permanent records as the worker sees fit. Paraffin, applied at smoking heat with a small brush, forms a neat, rigid and easily removable seal for such slides; after the initial use of the material the longitudinal paraffin strips may be flicked away easily from the cover-slip and the slide treated for permanency by Bridges' technique. For providing paraffin the apparatus described below is very handy.

A small 15 cc crucible is used as the melting pot; into this vessel, filled with paraffin, is suspended a crescent spiral of Chromel-A-22 resistance wire with a 22.5 ohm rheostat, a safety fuse, and a toggle switch in the circuit. After the initial adjustment of the rheostat a flip of the switch instantly produces smoking paraffin.



- A - rheostat
- B - fuse
- C - switch
- D - crucible
- E - two-hole rubber stopper
- F - attachment post

Bridges, Calvin B. The Examination of salivary chromosomes.

the resolving power and definition of the best microscopes. Aside from the use of very expensive and cumbersome ultra-

The detail present in salivary chromosomes extends in fineness beyond

violet apparatus with photography, the greatest amount of this detail can be seen by the following procedure, according to my experience. For objective and ocular I prefer 90 X apochromatic 1.40 N.A. with 10 X compensating ocular. Second choice is 120 X apochromatic objective with 10 X or 7 X ocular, and third choice is 90 X 1.30 N.A. with 10 X ocular. Oculars higher than 10 X seem inferior in results, though 12.5 X offers possibilities. For substage condenser 1.40 N.A. achromatic is best, and any achromatic is better than any aplanatic or other type of condenser. It is as imperative that the condenser be used oil-immersed and focused as it is for the objective to be so used. A front surfaced aluminized mirror (vapor deposited) is definitely superior to other mirrors and is more flexible in its setting angles than is the total-reflecting prism, which is otherwise the best. For examinations a binocular body with inclined ocular tubes is to be preferred. For camera-lucida drawings a monocular straight tube should be substituted.

Too much attention cannot be paid to the illumination of the chromosomes. For critical examinations one can employ a Bausch & Lomb thirty-nine Dollar lamp (nearly as good as the "research" lamp). The light is from a 6-volt, 18-ampere ribbon filament tungsten incandescent lamp, of projection type, with bayonet (not screw) base and brazed (not soldered) center contact. This lamp is run on alternated current transformed from 110 to 6 volts. Intensity of the light is controlled by a variable resistance, of not less than 175 ohms and not less than 1.5 amperes capacity, in the primary 110-volt circuit to the transformer. Use of this resistance is imperative for exact illumination and eye-fatigue. Also it increases the life of the lamp many times its rated life.

The light from the ribbon filament is brought to a sharp focus in air as near as practical to the front of the condensing lens. This is accomplished by turning the loop of the filament toward the back of the housing and moving the lamp mounting back in the housing and racking the condenser to its limit forward. After the filament is carefully aligned with the center of the condenser, an iris diaphragm is mounted in the plane of the image of the filament. The mounting support may be made as a right-angles wooden trough extending forward from the cylindrical lens casing below, and wired tightly to it. The iris is mounted to a smaller trough which slides in the support trough. To find the exact setting for the iris, put on the stage (condenser oiled) a slide and focus on it with the oil immersion objective. Next place the lamp with the field iris about 15 cm. from the mirror face and center the beam carefully on the mirror. With open field iris focus the edges of the ribbon filament sharply in the field by racking the condenser screw. Next close down the iris to about 2 mm. and slide it along its trough until its edge is perfectly sharp in focus. The iris carrier can thus be screwed permanently to its supporting trough. In critical use this field iris is closed to give a lighted area about half the diameter of the field of the ob-

adjustment of light under all conditions of filters, outside

jective, and its edge (as well as the ribbon at its midpoint) should be clearly in focus in the plane of the preparation. The iris beneath the condenser should then be closed down until the light haze in the marginal field narrows until it disappears by coinciding with the edge of the field iris image. This procedure does not use the full aperture to the condenser (approximately 0.6) but increases definition by contrast.

The carmine stain absorbs a maximum in the green at about 530. Hence to get greatest contrast a green filter should be used whose transmission is entirely within the absorption band of carmine. The best filter for maximum contrast is Wratten No. 62 (mercury Green). This is a dense filter and is recommended for cataloguing and being certain of the existence of the very faintest lines. For general use Wratten filter No. 61 (N) is the best, since it allows discrimination of the relative intensities of the lines through having a broader transmission (on both sides) than is the absorption band of carmine. Nearly as good as 61 for general use is 58A-B2 (dark). Filters 61 and 62 should both be obtained and none others are needed for acetocarmine work. They should be mounted as close to the substage condenser as possible, to avoid fading of a spot where light is concentrated.

In checking details of banding search for places where the chromosome (instead of lying lax) is stretched to two or four times its normal length. The stretch comes almost exclusively between bands and the bands are thus moved apart sufficiently to be distinctly resolvable as separate entities. Many apparently single bands are thus seen as doublets and an astonishing number of faint lines are brought to view. Final checking of bands is made by oblique light, cast along the axis of the chromosome. The oblique light is best obtained by sliding a black card partly over (below) the intake face of the substage condenser and as close to it as possible.

Microscopical examinations should always be done in a brilliantly and uniformly lighted room, never in a darkened room, cubicle or dark corner. When the general outside illumination and the microscope field exactly match in intensity there is a minimum of eye fatigue and dazzle by sudden entry of light through an expanded (dark-adapted) iris! Spotty sidelights and highlights should be cut out by a semi-cylindrical shield, made of Bristol board and wood and mounted on the microscope body. The interior of this shield and the tops of the objective and draw tubes should be painted neutral gray of very light color and flat (non-gloss) tone, instead of the customary glossy black.

It is recommended that camera-lucida drawings be made at a standard magnification of 5,000 diameters, which is large enough to represent the detail seen. This exact magnification can be obtained by trial of projection of a ruled stage-micrometer upon the drawing surface and adjustment of the length of path of projection until 10u on the stage micrometer becomes exactly 50 mm on the drawing board. The microscope and lamp can be mounted together on a board and this board raised above the

regular table by blocks to an amount exactly determined by trial. With 90 X objective and 30 X ocular and with mirror arm fully extended, the microscope base needs raising approximately 1-1/2 inches above the drawing level on the table.

Anthes, E.H. Critical illumination
for microscopic research

The need for a correctly designed illuminating unit for use with a re-

search microscope is no less than the need for carefully constructed optics. The Bausch & Lomb Research Lamp is specially designed to meet the requirements of the critical microscopist. Its source, enclosed in a light tight, yet well ventilated housing, consists of a 6 Volt 108 Watt Tungsten Ribbon Filament bulb which is used with a transformer on 110 Volt A.C. The bulb is of the prefocused type which eliminates tedious centering and alignment with the condensing system when lamps are changed. The lamp housing is fitted with a highly corrected condenser with iris diaphragm. The condenser can be focused by means of a lever. A support protruding from the front of the lamp house carries a water cooling coil and filter holder. Since it is necessary to control the intensity of the illumination to meet specific conditions a set of four neutral glass filters are supplied, having densities of 0.3, 0.6, 0.9 and 1.5 respectively. These can be used alone or in conjunction with Wratten or other filters. Best results are obtained when the source or Ribbon filament is focused sharply on the mirror of the microscope. The condenser of the microscope will then form a uniformly illuminated image of the front surface of the condensing system of the lamp in the plane of the object. When the specimen on the microscope is properly illuminated, the field of view will be completely and evenly filled with light if the object is viewed through the eyepiece. In addition, the light entering the microscope objective must completely fill the aperture of the objective. This may be checked by observing the back lens of the objective by removing the eyepiece and by viewing the back lens through a pin hole cap. It will be found that in order to secure the best results the condenser of the microscope should be carefully focused for each objective. In the case of an oil immersion lens, the back lens of the oil immersion should be completely filled with light, thus making certain that its numerical aperture is fully utilized. In the case of a dry objective (4 or 3 mm) the back lens need not be fully illuminated when working with lightly stained specimens as too much light tends to obliterate fine details in the specimen. It is suggested to reduce the iris diaphragm of the condenser of the microscope so that only 2/3 of the aperture of the back lens of such an objective is filled with light. When working with oil immersion lenses, care should be taken that the iris diaphragm in front of the condensing system of the research lamp is reduced as otherwise too much of the object is illuminated resulting in "glare". This field diaphragm should be wide open when low power objectives are employed. The full numerical aperture of an oil