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INTRODUCTION TO PHOTOGRAPHIC REGISTRATION OF THE MICROSCOPIC IMAGE

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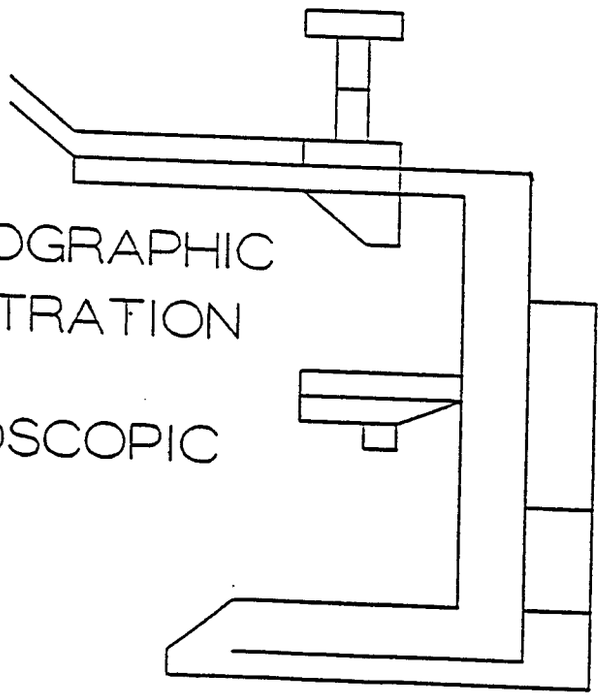

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I. PHOTOGRAPHY through MICROSCOPES

Introduction.

Photography is a beloved way of image registration, used for many kinds of reasons. Scientifically it offers many special advantages in association with fixation and conservation of observed images/events. Photomicrography is used in practically every field of science where a recorded image proves to be useful and a microscope is the fundamental tool. Some of the advantages are:

.there is a direct accessibility to information, like the minute microscopic details;

.photography produces a working document, e.g. to perform direct measurements on the pictures of features or their details;

.it offers the possibility to illustrate reports and articles.

.there is the good reliability, the relative great speed and ease of the procedure.

.it is a relatively cheep process to pass on (stored) data

.it increases the relevance and promises of a research record.

.in teaching it is easier to project a colour slide for class viewing than to have each individual gaze through the microscope and try to locate the feature under discussion;

Of course there are disadvantages as well. For example,in soil micromorphology a relatively great effort is needed to catch and exhibit different aspects of variability of features. All aspects connected to variability simply can not be shown on a single micrograph. One needs different magnifications and illuminations to display the type and nature of fabrics or the different existing forms of substances; one can show aspects of arrangement within or between fabrics and/or fabric elements. However, the advantages of photomicrography clearly prevail, regardless a few unfavourable consequences.

Almost any type of microscope can be used to make micrographs, no matter if it is a scarcely equipped hobby microscope or a research equipment with different advanced possibilities to enhance specimen visibility.

To deliver micrographs which are relevant and high in quality there are particular points to stress:

* One should be aware of the micromorphological topic (fabric or arrangement pattern) that is to be exhibited meaningful and prominent on the micrograph so systematic knowledge is required: to demonstrate the basic arrangement or the prominence of features mostly a weak magnification is chosen, but with regard to the basic arrangement pattern of domains (the b-fabrics) a moderate magnification in most cases will do the best.
* It is of highest importance that the photographic setup can be handled easily and fluently. Also all essential details which are visible through a microscope should be properly registered on the micrograph.

Consequently, a good familiarity with both elements of the system - camera + microscope - is required to be efficient in the use of it.

What ever the purpose of micrographs, never the recorded image can be better than the image produced in the microscope; although there is the possibility to enhance contrast photographically by film processing or enlargement, high resolution optics are essential for good results.

Subjects on micrography mostly are considered to be practical. However, there are subjects broadening the view on related aspects, which can signify the root of difficulties we can meet (background information). The topics on soil micrography can be grouped as:

#. matters with regard to acquisition of adequate samples: the collection and the preparation of micromorphological samples which includes drying, impregnation, hardening, sawing, polishing and grinding to thin sections

#. matters with regard to the microscope and microscopy: a.o the principles of light microscopy, handling the capabilities and limitations of optical components and the adjustment of the microscope, illuminators and lenses, optical methods to enhance the visibility: the illumination of the specimen.

#. matters with regard to the camera and photographic materials: the technical prospects linked to choices to be made on photographic materials, exposure and camera setup.

#. matters with regard to the observer: aspects concerning visual observation of; a.o. it may be interesting to draw attention to a few biological facts with regard to observation and perception of colour and contrast of morphological fabrics.

In the following chapters some subjects from the mentioned categories will be highlighted, but some will be left out, e.g. the categories with regard to the principles of light microscopy and the preparation of soil thin sections, as these topics are assumed to be sufficiently understood. We will concentrate on:

- (II) **Aspects of observation**: perception, some physical aspects of colours and of contrasts. (P.3).
- (III) **Photographic materials**: general remarks: colour materials, light quality/intensity. (P.7).
- (IV) **The processing of the photographs**, a few remarks. (P.10).
- (V) **Cameras used for photomicrography**. (P.11).
- (VI) **Exposure**, aspects during micrography: exposure meters, exposure readings, calibration of the light meter, judging exposures, record of exposures (P.13).
- (VII) **Conclusion**, (P.16).

Most of what is written here and more about photographic materials, cameras, and exposure can be found in a more extensive text in "PHOTOGRAPHY through the MICROSCOPE, a booklet from KODAK, Publication P-2, CAT 152 8371., by J.G.Delly published in 1980. "HUMAN INFORMATION PROCESSING", An Introduction To Psychology; Academic press, New York,etc. by LINSDAY,P.H. et al., can provide more extensive information about aspects of observation.

II. ASPECTS OF OBSERVATION
Perception of colours and contrasts.

Observation

Our eyes and nervous system together form a unit, the visual system. Besides perception of light the eyes are also equipped to convert light characteristics into an adequate neural signal; the nervous system can only transport and process neural signals. Perception and conversion happen in the rods and cones of the retina.
Depending on the perceived light as well as on effects from brain processes a specific visual sensation is evoked and judged in our mind; that is, our awareness is produced. Hence, physical and non-physical (psychological) factors that reciprocally take influence on each other together produce "the observation".

Observable details some times are very difficult to be clearly exhibited on a micrograph. This may be due to the photographic materials used, but it is also important to know that the awareness of the outside world is not singularly depending on measurable physical factors of the perceived light. Mentioned effects of the central nervous system such as enhancement of contrast, recognition from experience, etc. contribute to the creation of a "clear" morphologic impression by which objects or the parts they are constructed of are portrayed in our mind and appear similar or dissimilar to us.

Perception

Biological processes are not the issue here, so in the following a number of interesting facts are mentioned only and not worked out extensively; it is more an indication to a field of science where the reader can get some more background information. Extensive texts on this issue can be found a.o in: **Human Information Processing**, An introduction to psychology. Academic press, New york, etc. by LINSDAY, P.H et al.

From the total electro-magnetic radiation we experience just part of the waves as lights; the remaining like infra-red, micro-, radio waves, x-ray, etc. are invisible but have their own effects and are utilized.

Major aspects of lights in connection with perception are the quality and brightness or intensity. The rods and cones of the retina are the sensitive elements in our eyes. Through a photochemical reaction (not to be treated here) the rods and cones disclose the radiation properties and convert them into an adequate signal that can be transported and processed by the nervous system.
The rods typically register only intensity levels (processed as grey levels lacking any colour). They are dominantly involved in observations with low intensity levels of light: e.g. in the twilight/night.
The cones register both the quality and the intensity of light beams. There are three types of cones, which operate two by two; each type is specifically (only) sensitive to particular wavelengths.

Our visual system is a unit that is perfectly equipped to detect relative values: most individuals can perceive, distinguish by comparison, understand and classify (judge) the quality and intensity of lights reaching our eye. The visual system, however, is not equipped adequately to measure, verify and confirm absolute values of the detected light. Detected differences in colour and brightness help to distinguish objects from each other: fabrics and fabric elements in micromorphology.

Contours are very dominant in our visual perception: e.g. when drawing we begin to sketch the outlines. We see contours when a contrast is there: this can be evoked by differences in brightness (intensity) or in the colour between adjacent areas.

The varying physical and psychological factors contribute to (or even guarantee) the best possible perception and observation at any moment. This means that our awareness is not necessarily exactly the same as reality, but thanks to the way our system operates it serves us to the utmost in the majority of situations that each individual could get into.

Every individual is unique in perception as well as in processing of perceived light. As known there are individual differences in:
- the sensitivity of the eyes toward physical factors coming from the outside world, such as the level and quality of light.
- psychological processes: enhancement of contrasts and awareness.

a. visual adaptation, the ability of the eye to adjust its sensitivity to changing levels of illuminations, e.g. if one enters a room with a low light level from outside it will be dark, but with the passage of time we will see more in the room: the result of "adaptation" to the lower level.

b. enhancement of contrasts. Differences at the border area help to distinguish different objects and to recognize patterns. By contrast enhancement -(sensory sharpening)- the visibility of the boundaries is increased: objects can be located better, even if there is not much difference between the object and the surroundings in quality, saturation or intensity. For example, the result of "induced contrast" is that boundaries (contours) are observed more clear due to suppression of the similar colour in the adjacent field by lateral inhibition of receptor cells. This neural process creates an artificial, stronger contrast between the two fields so they are more obvious to distinguish from one another.

From here on we will concentrate on some physical aspects of perception: of colours and of contrasts

Some physical aspects of perceived colours.

The quality of a light beam is associated with but one specific wavelength, the colour of monochromatic light. Note, that in normal speech the term "a colour" covers a range of wavelengths which can be described separately: there are many shades of a colour, (e.g. green colours).

The colours that we see a.o. depend upon of the wavelengths striking our eye: the light quality. It can concern the light that has been reflected by an object (e.g. during field descriptions of a profile) or the light after passage through a material (e.g. micromorphology). Light can penetrate materials or it is reflected. When lights penetrate into materials the intensity will accordingly go down, because of absorption. Hence, very thin slices of materials, like soil thin sections, will let through enough light to be used as microscopic slides.

. Absorption can vary from very weak (clear glass or water) to very strong (opaque minerals) when even the very thinnest slices remain black.
. Absorbtion can be selective if specific light beams (a narrow range of wave lengths) are absorbed stronger than the rest, so the quality of white light passing through is changed to some colour. For example green glass absorbs blue and red beams; the remainder is green.
. If absorption is continuous a considerably large range of wavelengths is absorbed; practically only the intensity of white light goes down without changing the light quality. (see density filters, p.10).
. Total absorbtion or the absence of light, result in observation of black.

The spectrum

It is important to note that white light is produced through a mixture of monochromatic colours: white is not a (monochromatic) colour! The different colours building up white light can easily be shown. Passing a bundle of white light through a prism, the spectrum of the composing visible wavelengths is produced: the different wavelengths are visualised in a sequence of light bands which are changing their colour from blue to red as wavelengths become shorter. According to this layout every separate (monochromatic) beam (of one specific colour) has a specific refraction! In the spectrum some of wavelengths that occur close together are forming a colour band, meaning that the almost similar wavelengths have almost similar refractions and some of the similar waves evoke almost similar visual sensation in the same situation.
However, looking at the spectrum of pure lights produced by the prism some colours are absent: brown, purple and pink. These colours, like white light, also must result of some thing other than a simple monochrome light.

If we look at the wavelengths at both sides of the spectrum the visual impression will be the same: deep blue changes to violet, to black, respectively red changing (via purple) to black: infra-red, etc. which we can not observe. Observation of black means the lack of any visible light coming to the eye: nothing is observed at all! Black is not a colour!
Now if we take the line of colours and draw it as a circle and see to it that the complementary colours are direct opposite each other across the diameter of the circle than we have constructed the colour circle.

Colours in the colour circle

If we project two light beams across each other on a screen we will see just one new colour formed from the mixture. Some colours, when they are mixed, leave a colourless grey: both of the colours cancel. Such colours are said to be complementary pairs.

Three characteristics of a light are needed to characterize and/or represent a colour, no more no less. Also for representation in the colour circle these attributes are required: HUE, BRIGHTNESS AND SATURATION.
Hue covers the normal meaning of the term "colour" or quality: it changes with the wavelength.
Brightness in physical sense is most related to light intensity (value).
Saturation - the richness of colour - refers to the relative amount of pure monochromatic light that must be mixed with white light to produce the perceived colour: it can vary without a change of the colour.

Note: The colour circle applies to (additive) mixtures of lights beams, not to (subtractive) mixtures of paints!

The colour circle shows colour names and approximate locations for the wavelengths of lights viewed in the spectrum. Complementary pairs are situated opposite each other: yellow/blue and green/red. The region marked "purples" can not be produced by monochromatic light (the opposite of magenta, a purple, is cyan). On the colour circle the brightness dimension does not show up. The centre of the colour circle represents grey, a point with no colour, referring to a general class of brightness perceptions that range from black (very low brightness) through the greys to white, (a very high brightness).

The colour of wavelengths (radiation) is observed by projecting monochromatic beams on a white field.
The colour of paints is observed by shining white light on it.

The mixing of colours.

Three main colours have been established: red, yellow and blue. By mixing colours in a correct balance regarding quality and intensity, practically every colour awareness can be produced including white. Interestingly the mixing of (monochromatic) wavelengths produces an effect that differs from the mixing of paints.

a. **The mixing of wavelengths.** When two monochromatic wavelengths are mixed together by projection across each other we do not see two colours. Rather we see one new colour formed from the mixture. Once combined, we are not capable to determine the original set of colours that went into the mixture. In addition, when complementary (contrasting) monochromatic light beams are mixed in the proper intensity they can neutralise each other totally, meaning that no colour is generated at all, or that all colour is cancelled. For example, monochromatic yellow-red of 600 nm and blueish-green of 490 nm beams (complementary pairs), when mixed by projection across each other, and the relative amounts of the two lights are adjusted just right, the colours cancel leaving a colourless grey.

b. **The mixing of paints.** Mixing blue and yellow paints of the same colours as before would result in green as every body knows.

Why these differences? The different effects result from the fact that while mixing monochromatic light the perceived qualities are neurally processed and treated as an addition of the light beams that contribute to the observation. In the second case, mixing paints, most of the beams composing white light are absorbed by the paint particles and only a few are reflected. Thus there is a subtraction of light beams from white light and depending on the paint particles the beams that reflect to our eye will be yellow or blue. The brains translation of the countless random reflections (of the two colours) of all the tiny yellow and blue particles which can not be distinguished individually is green.

Some physical aspects regarding visual contrasts.

To distinguish soil morphologic objects from each other, or for micrography of the material we need any kind of contrast between the objects. Regarding our perception two types of contrast are of importance:

- the brightness or light intensity levels.
- the colour differences (colour contrast).

Brightness contrast. The most direct physical correlate of brightness is the intensity (of colour or of the grey level when colour is deficient:). Every colour has an intensity level that is related to the saturation of the colour. Absorption means decrease of light intensity. Saturation refers to the relative amount of pure monochromatic light that must be mixed with white light to produce the perceived colour.

It can happen that two objects with very different colours have identical grey levels, so they can not be distinguished from each other on brightness only, e.g. on black and white photographs; colour is needed.

Colour contrast. Every object or structural fabric has a specific overall appearance of the colour. Colours are very easy to distinguish among each other, even on the slightest differences in hue or chroma or brightness. (Note phenomena of induced contrast as shortly mentioned.) Colours can only be perceived if they have a sufficiently high intensity level. However with changing intensity levels there is a shift in our awareness of the colours: in bright light a red may appear brighter than a blue, but in dim light the same red can be dimmer than the blue. In spite of that when we compare some object with the Munsell colour chart there will be no problem as the circumstances are the same for both the object and the card.

The Munsell system, an atlas of charts with colour samples, is developed to easily classify colours of objects (under similar conditions) by a direct comparison with a standard; a method strongly oriented on perception. The chart is a great tool to estimate the colour of soil materials by comparison during field work.
It is produced according to defined "tones" of colour: "the hue", the colour saturation or the "chroma", and the grey level: the "value". In the atlas adjoining samples are placed on equal distances (in the sense of perception) as good as possible.

The form of an atlas shows that the Munsell system was not created to compare or characterize light setup. However, as there is no better alternative, at least it is some thing, and so inspite of the reservations one should have it is also used in micromorphology to describe the colour of the fine mass using a standard.

III. PHOTOGRAPHIC MATERIALS.

a. General remarks.

Before one starts to take micrographs the correct film must be chosen. A correct choice depend on the properties of the film, those of the subject itself and finally of the purpose of the micrograph.
For example with regard to contrasting properties of the film the question of what is essential to be exhibited or measured can influence the choice between black and white or colour film; the film size and film speed are important as well, and there are even more properties to consider.

The expected degree of enlargement also plays a role in the choice of photographic material. As long as the reproduced enlargements are not so strong there will be no problems e.g. with the granularity, but if it is expected that strong enlargements will be required in a later stadium it could be wise to use negative film of a larger size (4"x 5") for the documentation instead of the normal film of 24 mm x 36 mm.

Films are of four major kinds: reversal films (diapositive) and negative films, colour and black and white film. Black and white reversal films are seldom used. The colours of diapositives are comparable to those in the existing world. The negatives display colours that are complementary to those of the subject. Especially when using negative film we should know some of the characteristics of the material, like its colour sensitivity, effective speed, granularity, contrast:
The colour sensitivity of film materials. This characteristic is related to the response of a film or its sensitivity to spectral colours. Both black and white and colour films have this characteristic.
Colour sensitivity is a fixed property which can not be altered during processing the film, in contrast with other characteristics.

Colour film materials are balanced during manufacturing for artificial light of tungsten lamps or for day light. They have three differently sensitive layers: for red, yellow and blue: the three main colours that can combine to white or any other colour.
Interestingly black and white films can specifically be manufactured to cutoff part of the spectral beams. They are classified as:
.sensitive to blue, in which case only blue and ultra violet radiation is registered,
.orthochromatic films, having the sensitivity extended into the green,

.panchromatic films, being sensitive to (= will register) all visible colours (wavelengths),
.infrared sensitive, recording all visible light plus infrared radiation.
The effective speed. Effective speed of a film material is related to the sensitivity toward light intensity. Normally the film speed is expressed by an ISO number showing the arithmetical speed (in ASA numbers) and the logarithmical speed in din numbers (6 to 39), whereby 12 Din = 12 Asa. Using the arithmetic ASA designation a film of ISO 400 is twice as fast as a film rated ISO 200. Note that increasing a Din number by three, doubles the sensitivity.

The rated speed for a film given on an instruction sheet supplied with the film only applies when the film is developed according to the recommendations. Deviation from the development procedure can have a clear effect on speed and contrast (graininess): the developer or the development time can be manipulated. A useful use of this phenomenon requires some experimentation to establish a exposure index. A film of moderate to medium contrast will usually be capable of the widest development latitude.
The granularity. Granularity determines the reproduction of contrast in film materials. Contrast can vary from soft to very hard. Two aspects or factors should be paid attention to: the graininess and the distribution. . The size aspect of the graininess of photographic materials varies from very fine to very coarse grained material such as x ray film. Generally one could state that the finer the grains, the lower the sensibility of the material, but the better the contrast and so the better the resolving power. A fine grained film increases the probability of good image quality!
. The graininess is not only related to size of silver grains produced in a film during its development but also to the distribution of the grains in the emulsion. The graininess as displayed in a film depend on the type of developer used and on development time, so it can be manipulated: a fine-grain developer may produce less graininess, but speed and contrast are reduced.
The resolving power, contrast and film speed. Resolving power of films refer to the ability of a photographic materials to register fine structural details separately (distinguishably) in colour or in grey tones. It is defined as the number of line pairs per millimetre that can be recognized as separate spaces and lines in a photograph.
Generally, slow films have finer grains than fast film. Fine grain film has the advantage that the probability of a good image quality is high, but it is no necessity to achieve high resolution in photography. A film of high resolving power will show an image more effectively than one of coarse grain or one with low resolving power.

Note! The detail or resolution of the photographic image can be improved by enlargement, only if the details had been portrayed by the microscope so they could be present in the photographic image. A high resolution in the image can only be achieved using a quality microscope efficiently. In other words: a good microscope with a good resolution of the optics comes before photographic refinement.

Black and white films generally offer a great variety in contrast (from 25 to 300 lines/mm). As a rule the colour films have considerable lower resolving power. However, this is easily compensated by the extra information that is offered by colour perception, e.g.colour quality, saturation.
When choosing a film a general policy is that if the image has a relatively low contrast a film of high contrast is used: when the image has a high contrast films of low contrast give the best results (details).
In general one could state that: the lower the effective speed, the better the resolving power or higher contrast of the film material.

b. Colour materials, registration, light quality and intensity

Mostly micrography of soil materials in colour is almost a must: many details can only be detected due to colour contrast. However, ultimately the microscope in combination with the illumination should produce the best possible image of the specimen and a photomicrograph is the record of this image on a photographic material. If colour film is used there are some aspects that deserve some extra attention:

The registration ability, that is, the comparison of the registered colours with the observed colours of a specimen.

The quality of colours that will be registered on the film strongly depends on a number of factors, especially the quality of the illumination and the amount of light, fixed by the light intensity and the exposure time.

There is the colour temperature of the film, the required quality of the light during film exposure, which is decided in manufacture of the film; the needed balance between blue and the red.

For productive photography the light source requirements must be fulfilled.

Film materials are produced either for daylight or for artificial light (tungsten-balanced). Consequently, the production of films aim at fixed but different light qualities.

Hence, the use of a daylight film on a microscope with artificial illumination will require colour correction filters to achieve or at least approach acceptable light quality.

For artificial light -(TUNGSTEN)- different indications are used : type A, indicate the type that was balanced at 3400 K; type B at 3200 K. Professional films are indicated either type L or type S.

The type L stands for long exposure film and is balanced for 3200 K: relatively long exposure times (1/10 second to 60 seconds) are possible.

Type S is balanced for daylight illumination: it is used when short exposure times of 1/10 seconds or less are expected.

Daylight films balance at 5500 K (average sunlight or electronic flash).

The amount and the quality of illumination. The successful registration of a colour a.o. depend on the appropriate amount of illumination. Too much light decreases both types of contrast.

In regular photography there are two possibilities to regulate the amount of light passing through the lens: exposure time and diaphragm. In modern photomicrography using cameras without integral lenses the diaphragm f the microscope is constant, so there is but one possibility left for regulation of the amount of light: shutter speed in combination with grey filters.

Working with artificial light of the microscope, the proper light quality to be used for photography is only achieved at one specific level of the transformer if no colour balance filters are used. And so, to meet the requirements of the film the transformator has to be set and kept on a prescribed amperage or voltage level.

For normal microscopic observations the produced light level is too high to the eye. It can best be brought down for observation by the use of grey filters which do not alter the light quality. (remove for exposure).

Note that there are different kinds of bulbs that can be used for photomicrography: e.g.(6v/30w, 12v/60w, 12v/100w, 6v/15w). Each requires a specific transformer and there is a relation between colour temperature and amperage. This means that for every set up it must be checked at what amperage or voltage the required colour temperature can be reached.

Note also that when light intensity is too high to our eyes this reduces the actual observation of details (focusing and registration of the boundaries of small or vague structural features) as well as the concentration.

The resolving power of colour film. The resolving power of colour film is many times lower than in black and white. This is compensated on photographs thanks to the contrasting effects that colours produce in our perception / observation.

Coloured objects can be photographed best in colour.If black and white film is to be used, a film should be chosen that gives many shades of grey, the "translation of the colours.

c. Filters.

Ultimately the microscope and the illumination should produce the best achievable image to be recorded on the photographic material. If the colour temoperature of the illumination differs from that for which a colour film is balanced it can be adjusted by light balancing (colour) filters. If the brightness is too high neutral density filters can be used to reduce the brightness.

A light source with a colour temperature that is lower as required should be "raised" to the correct temperature with one or more blueish light balancing filters. Different filters are available. Each filter will raise colour temperature by a definite value: in fact the filters modify the illumination and simulate a higher colour temperature. A colour test-exposure series with the different available filters eventually in combination will be necessary to find the best colour balance to used in subsequent micrography with this film type.

Yellowish light balancing filters bring down the colour temperature. Also here a number of different filters are available, each with a shift value of its own.

Daylight colour films can be used in photomicrography if the correct filters are placed in the light beam, adjusting the light to daylight quality or if a source of artificial light of daylight quality is used the filters can be left out.

Neutral density filters. This type of filters have a continuous absorption, so practically only the light intensity decreases. Also here there is a range of filters with different densities. The main use of these filters is to prevent over exposure during photography but also to reduce visual image brightness when it is too high for comfortable watching (remove before taking the photograph to avoid lang exposure times and the reciprocity effect). Evaporated metal on glass is one of the best forms of neutral density filter.

IV. PROCESSING OF PHOTOGRAPHS.

The production of prints from soil materials is an everlasting problem. For colour prints one has to rely on the photographic laboratory. Normally there is no standard reference for soil materials at the processing centre. Consequently it is not clear to the personel which filters should be used during the reproduction process to achieve colour corrections if needed.

The best response to this problem is to keep strictly to the light quality prescribed for the film while taking the micrograph. If this colour balance level is told to the man working in the processing laboratory he can make his choice on filters to produce an excellent print. A diapositive if sent with the film, can also contribute to overcome such a problem to some extent.

V. CAMERAS used for photomicrography.

Camera and microscope are united in a system in order to produce photomicrographs. The microscope is the image forming part of the system and the place where the illumination is controlled. The camera is only the means for recording the image that is produced by the microscope.
It is of highest importance that the image produced in the microscope is of the best possible quality, thus the best possible basis is created to produce an excellent micrograph.

In the following some remarks on cameras are given using a broad classification.

a. Cameras with integral lenses.

Photomicrographs of the most simple kind can be produced by a conventional camera over a microscope. This can be a cheap fixed focus or 35 mm camera with the lens as an integral part of the camera that can not be removed easily.
A fixed focus camera is the simplest with one shutter speed and one aperture. The more expensive the type of camera the more shutter speeds and regulations for distance and aperture settings to control the exposure. Generally the aperture settings (f-numbers) on the camera do <u>not</u> control exposure, they have no effect on image brightness; the largest setting should be used.

A microscope when focused with a normal relaxed eye the image is considered to be at infinity. that is why the distance setting of the camera should be at infinity. Hence, the image will be in focus on the film plane when the camera is placed with its lens at the eye point. This position can be determined by holding a piece of paper on top of the eye piece, then slowly raising the paper until the bright circle that appears on the paper reaches its smallest transsection: the eye point.

The camera can be held over the microscope by any available means: a wooden or metal vertical stand of own construction, a laboratory ring stand, a camera tripod, etc. In any case the support should hold the camera firmly in the correct position. At the same time you should still be able to swing the camera out of the way to be able to look into the microscope.

b. Cameras without integral lenses.

Reflex cameras. These can be adapted to be used over the microscope. Often adapters are available from the firms that manufacture the camera. The normal lens is removed from the camera replaced by extension tubes on the lens position. An adapter ring, containing the microscope eyepiece, is fastened in the extension tube. The whole assembly of camera, tubes and adapter is placed on the microscope, fitting the eyepiece and the adapter ring into the draw tube of the microscope. This assembly is attached to a rigid stand that eventually is supported independently from the microscope.

The image to be photographed is focused with the knob on the microscope while looking through the cameras viewfinder. Focusing on a ground glass within the view finder is alright for lower magnification. Critical focus of fine detail is best achieved if a clear area is present on the ground-glass near the centre and with cross hairs, providing that the aerial image is focused in the glass and allow parallax focusing.

Bellows extension camera. Bellows are normally used in close up photography and on a reflex camera over a microscope. The great advantage is that it can be adjusted, providing that magnification and size of recorded field can be varied for image composition.

c. Photomicrographic cameras.

Although almost any camera can be used, it is of advantage to use a camera that is specifically constructed for photomicrography.
There are several commercial cameras designed for this purpose:
The so called "eyepiece" or "photomicrographic attachment" cameras are the most popular cameras of this group, having in common a beam splitter eyepiece. One can view and focus the image by means of this auxiliary telescopic eyepiece.
Cameras of this type usually are equipped with 35 mm magazines, although some use sheet films. Only the central part of the field of view is photographed
In the following a short classification of eyepiece and attachment cameras:

· 35 mm eyepiece Cameras. The film plane in a 35 mm eyepiece camera is in a fixed position, far enough from the microscope eyepiece, to alow only the central part of the field of view to be recorded. This position avoids the out of focus peripheral area of the field of view to be recorded; the area is existing because of a curvature of the field of view which is inherent in many microscope objectives.
This type of camera requires little room when not in use and it can be set up quickly when needed. Today, these generally reliable attachment cameras are offered with built-in automatic exposure devices and frequently with an self-operating film advance as well

· Sheet-film eyepiece cameras. When sheet-film cameras are of the eyepiece type they often reproduce visual microscope magnification because the distance between eyepiece and film can be fixed at 10 inches.
These cameras often have a ground-glass back. Composition and focusing of the image to be recorded is facilitated in this way.
The sheets are usually 4x5 inches; the sheets can be developed separately.

Adapter backs are available to convert the sheet film cameras to the use of roll film or for instant materials.
These larger format cameras spread the same amount of light as used for 35 mm films over a much larger area. Consequently the exposure times are longer. (see choice of film material).

· Trinocular attachment cameras. A trinocular microscope has a binocular arrangement for visual work and a third tube for micrography. The construction is such that the image seen in the microscope will also be sharp in the camera. The side telescope is lacking!
Sheet-film and 35 mm film backs can be interchanged. Often a special field of view eyepiece is supplied containing a graticule to indicate the area of field that will be recorded.
Depending on the design simultaneous viewing and photography is possible or not. With a beam splitter arrangement similar to the eyepiece cameras a constant observation is possible, and so the photography of moving objects.

Bellows attachment cameras. Cameras with adjustable bellows are capable of a complete and continuous range of magnifications because the distance between eyepiece and film is adjustable.

Many microscopists consider bellows cameras that accept large film size as the best for high-quality photomicrography. The shutter of such cameras normally contains a wide range of exposure times, a ground-glass screen for composition and focusing. Film size is usual 4x5 inch to allow highest magnifications and great field sizes.
The cameras are mounted on a rigid stand to avoid vibration (possible blurred image). Particularly with long exposure times stability is vital. Vibration in a system can result from (microscopic) manipulations of any kind, setting the mechanical shutter, or transmission through the stand. It is good to wait for the vibration to cease than make the actual exposure.

Also it is advised to use a cable release, this is a better security against vibration. The apparatus must be situated on a stable table, eventually on vibration absorbing materials.

d. Photomicroscopes.

These instruments are specifically designed for photomicrography during microscopical work. Especially when frequent documentation is demanded an uncomplicated handling of the photographic recording is essential. To achieve that goal the photographic mechanism is built in the microscope rather than attached to it as an accessory and electronic automation has been introduced.

e. Choice of a camera.

It was shown that outfits for micrography can reach from very simple to very advanced. It is clear that production of photomicrographs does not necessarily require the most expensive outfit. An anticipated photo work load, the variety of microscopical tasks, costs, but also preference and experience etc. can be used to make the correct decision if a choice must be made: principally all the photo systems are capable of producing high quality photomicrographs.

VI. THE EXPOSURE: aspects during micrography.

Exposure Meters. In the production of photomicrographs artificial light is used. A certain limited amount of light is needed to record an image of a particular brightness. Exposure is the result of that limited amount of light, controlled by light intensity and exposure time.

The intensity (the image brightness) getting through is recorded by the exposure meter. The intensity value is used to find the exposure time. Note that correct exposure times partly depend on the film speed.

The exposure meter should be situated some where in the path of the microscopic beams (see exposure readings). It should be sensitive to low to very low light levels because through the microscope the brightness of images can be quite low.

On the microscope there is a fixed diaphragm. So, except when using a camera with integral lenses or grey filters, photomicrography indeed has but one option to control the amount of light: regulation of the exposure time. The correct setting can be determined automatically as is the case in advanced equipment, but manual operation even when using a normal exposure meter is also very well practicable.

Exposure readings.

The readings can be made in various locations: at the film plane of the camera; between the film plane and the eye point of the ocular; in the eyepiece tube of the microscope. At the film plane is the best position, while one measures the same brightness as would be recorded on the film. For example on the ground-glass screen of a sheet film this location is accessible before the film holder is inserted. Readings in other locations will differ from that on the ground-glass but that does not really matter provided that they are taken at the same location as the test film: on the job light readings should always be made at the same spot.

Generally there are two possibilities to measure the exposure time. The first is to make a spot analysis. This motte works perfectly as long as the image has average brightness. Dense areas may encounter underexposure or high light areas may be washed out. If a good rendition of detail is required in the darker areas one can deliberately prefer this method. The second method is to make an analysis of the whole field. This ensures a good average brightness on which the reading is made. This method should be used especially with reversal colour film while the exposure time is dependent on the brightest parts, essentially the back ground brightness.

The heterogeneous character of soil materials hinders the determination of exact exposure times. To obviate this problem one can experiment with different exposure times. This is especially true with respect to diapositive slides as the best quality of a projection strongly depend on the circumstances during projection: the slide itself, the projector and the distance to the screen affect the quality of the projection, etc. To guarantee a correctly exposed photograph it is recommended to take three pictures including one above and one below the previously assumed best exposure time, especially when taking micrographs is not a daily routine.

Generally there are a few things to note:
-Follow the instructions of the producer of the film carefully.
-Diapositive slides need an exposure time twice as long as the one for negative film. Exposure times largely depend on the background brightness.
-Under darkfield illuminations, e.g. crossed polarized light, exposure times are twice as long compared to normal transmitted light conditions.
-Colour filters influence the quality of the light, grey filters do not. So, during photography all colour filters should be taken out of the light beam unless they are prescribed by the producer of the film material.
-The reciprocity effect. According to the reciprocity law: "...the product of the intensity of the illumination falling on a film and the exposure time equals the amount of exposure...". Consequently, an exposure of 1/60 second at f/11 is equivalent to an exposure of 1/30 second at f/16.

Each emulsion has its greatest response within a particular range of illumination values. Out side this range the response changes. Far outside the normal range, the effect of reciprocity-law failure can be regarded as under exposure. The result is a change in contrast in black and white films and a change in colour balance in colour films. This means that the reciprocity law does not hold for very short or very lang exposure times! Because colour film has three sensitive layers, each can be affected differently. Colour balance correction become necessary.

The exposure time. Light amounts. Micrographs of soil materials are taken in transmitted light or in reflected light using different illumination technics. Each condition has a corresponding optimum aperture setting, whereby darkfield illuminations take the double amount of light (e.g. a calibration at 2.8 for bright field illumination corresponds with 1.4 for dark field illumination). The exposure time is then read from the meter on different locations for these two settings.

Calibration of the light meter.

Regardless of the method used to establish exposure time, it is important to calibrate the exposure meter to conform to the method to be used and to assure consistent, accurate readings.

How to do the calibration? The light intensity getting through a thin section is shown on the meter and recorded, although it is meaningless at that moment. Then the roll is exposed at all shutter speeds. After developing the best exposure is selected. Now the recorded intensity value is turned into a window of the meter. The shutter speed with the best result

should now be located opposite or near by a diaphragm (probably the fixed value of the microscope): the calibrated setting where shutter speeds are to be read in the future. To be sure about the correct setting a number of test series are produced to confirm the calibration value.

The calibration values defined above are determined by experiment. Users are advised not to neglect this step of experimentation with their own microscope to obtain the optimal exposure times for their system.

Judging Exposures

When the subject is shown to best advantage (on negative material or on reversal film) a good photomicrograph has been produced. However, many factors and mistakes can affect image quality:

-The optics. Optics can be of low quality, the optics can be dirty, the adjustment and alignment of illumination and optics can hamper;

-The specimen. Some thing may be wrong with the specimen itself, e.g. an inaccurately fixed thin section, dust or greasy dirt of finger prints;

-The use of filters. The incorrect use of filters can reduce contrast or detail. When using colour film, also the colour balance will be affected in a negative sense.

-The exposure latitude of films. Reversal film has a very narrow exposure latitude, so it is easier to judge correct exposure on reversal material than on negative materials, which perform considerable more exposure latitude than reversal colour films do, so they are more difficult to judge what is the best exposure.

. Incorrect exposure times are promptly obvious: over exposure will wash out the light areas, with a loss of highlight detail. under exposure will display a widespread darkened appearing, loosing the details in the darkest areas. A good exposure will show detail in all areas where detail was spotted in the thin section.

Generally one looks at the darker areas which are represented light on the negative. Under exposed film will display little detail in these areas.

With negative materials overexposure can be permitted more than underexposure, but significant overexposure can cause an un acceptable loss of detail especially in the light areas of the negative.

-The image sharpness. Lack of image sharpness can have several causes:

. the microscope is too old and worn out and there is some slippage in the fine focus adjustment of the apparatus (sharpness changes after critical focusing while watching).

. the camera shutter is activated too hard or too fast: the image is indistinct or blurred as the result of vibration. The effect will be more obvious at high magnification and with long exposure times.

If the cause can not be traced and eliminated the alterative is to mount the photographic device on shock absorbers.

. the surfaces of optics may have been touched by accident. Dirty optics can cause a hazy image, so inspect all essential surfaces before taking the micrographs and eventually clean them. If necessary use a lens cleaner, but NEVER USE ALCOHOL to clean the lenses as alcohol is a solvent for the cementing medium in the objective and can be harmful.

-Inappropriate illumination. There may be several causes:

. Substage condenser and objective should be aligned satisfactorily;

. The light source and the illuminator within it should be aligned with the microscope to secure an even illumination. If not the micrographs will be darker on one side. (Kohler illumination should be used). The effect of improper illumination is articulated best with low magnifications having a relatively large field of view.

-Too low contrast in the image. Basically there are four grounds:
1.The substage diaphragm is not closed far enough, thus creating flush.
2.An inappropriate filter is used and is reducing the contrast.
Colour compensating filters cut down a larger or smaller range (partial absorbtion) of wavelengths, so they should be used with care as this clearly affects the recording of colour and brightness.
3.The subject has a very low contrast of its own. If desired favourable the contrast can be enhanced e.g. by using different illuminations, filters, etc. The low contrast may be a typical feature and be presented as it is.
4.the field diaphragm may be opened too far.

A record of exposures

If photomicrographs are made regularly it is advisable to make a record of the conditions. Giving the details in this record it is not too difficult to duplicate a similar set up for similar features in the future or to rephotograph a specimen.

This information is recorded best in a notebook. For soil micromorphology data like slide nr., thin section number, location on the thin section, used filters, the magnification used, exposure reading and exposure time, finally a description (the purpose) of the micrograph. If the record is organized for every separate profile the profile number, its classification, horizon where the feature occur can be added as well. In this case one can also consider to use a record book composed of loose sheets providing the possibility to add or insert new sheets. If more micrograph are requested at a later date they can be recorded organically.

VII. CONCLUSION.

If you encounter the situation that you are not satisfied with the results in spite of careful choice and accurate handling of both the photographic materials and the system, try a film of a different trade mark or of an other speed. Whatever is decided, one should always carefully follow the conditions required with regard to the film and review conditions that affect the exposure in one way or the other.
Only in last instance one should try to use colour correction filters.

Undesired effects on colour balance can be the result of incorrect use of optics, incorrect film storage or film processing or the reciprocity effect. When the shift of colours is not too pronounced imbalances can be corrected during the printing of colour negative materials .

If image brightness in the observation eyepiece is too low, it is necessary to resort a light source of higher intensity to obtain a bright image on the film: especially when black and white film is used this can be done easily without thinking of colour balance.

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