CHAPTER - 2

Work is Worship.

MATERIALS AND METHODS:

2.1. SELECTION OF THE VEGETATION FOR SURVEY AND COLLECTION

A careful study of all existing and available literature of the region was made prior to visiting the different areas for field study and collection. At each stage beginning from selection of vegetation to the mounting special care had to be taken for proper and effective documentation.

A large number of places in representing all the three hilly subdivisions for Darjeeling and all the districts for Sikkim with the altitudinal range from 900 m to 4200m were visited in different seasons for the collection of all relevant field data including the time of flowering, fruiting, leaf fall, break of dormancy, quadrat study etc. Each trip usually included a trek along the altitudinal gradient. In the Darjeeling subdivision the entire locality from Sonada (2000m), Ghoom - Jorebunglow- Jalapahar - Katakpahar (2300–2350m), Jorebunglow - Rangarung - 3rd Mile-Rungli - Rungloit - Takdah (2200-1300m), 3rd mile - Mungpoo (2100-1300), Ghoom - Senchal - Tiger Hill (2300-2400- 500m), Sukhia Pokhri - Maney Bhangyang - Megma (2200-2000-2400m). Megma - Tonglu (2400-3000m), Tonglu – Garibans - Sandakphu (3000-2800-3636m), Sandakphu – Sabargam - Phalut (3636-3300-3700), Sandakphu - Samanden –Kalikhola – Ramam – Srikhola - Rimbick (3636-2400-2400-1900-2200m), Rimbick - Lodhoma (2200-1300m), Darjeeling – Lebong - Ging (2100-1900-1700); Lebong – Pattabong (1900-1400m), Rungbul – Moonda Kotee (1950-1600 m) in the Kurseong subdivision, Dilaram - Kurseong (1700-1650m), Kurseong - St. Mary's - Upper Mamring - Lower Mamring (1650-2000-1700–1300m), Mirik - Manjua (1900-1000m); in the Kalimpong sub-division Lopchu - Peshok - Rayang - Kalimpong (1500-900-300-1350m), Kalimpong – Pedong (1350-1400 m), Kalimpong - Rashet (1350-1500), Kafer – Lolaygaon - Munsong (1200-1350-1900m), Latpunchor (1200m and above), Kalimpong – Labha - Kolbong (1350-1450-2300m); Labha - Panmkhasari - Khampong (1450-2400-2000m), Rhenok - Rashet (1400-2500m); Rechela (1400-3160 m), Thosum (1350m-3040 m). In Sikkim in the west district Gazing – Pemayangste - Yoksum (1550-1900-1700m), Yoksum – Bakhim - Djongri (1700-2300-4000m), Djongri - Thangsing (4000-3900m), Djongri - Olothang (4000-3900m), Djongri – Gamothang - Migothang (4000-3750-3900m); in the south district Temi –Damthang - Tedong (1600-2100-2900m); Singtam – Temi – Kyosing - Rabong la (700-1600-1700-2100m), Jorathang – Namchi - Sombaray (300-1700-1200m), in the north district Mangan -Chungthang - Lachung (2100-1600-3000m), Lachung - Yumthang (3200-

3900m), Lachung -Kathak (3000-4100m); Chungthang – Lachen - Thangu (2100-3300-4000); Zakophyak – Katoa (3500-4200m), Memang - Zemu (3700-4200m); in the east district Pangolokha - Menmenchu (2800-3700m), Gangtok - Penlong (1650-2100m), Gangtok - Lagyap (1650-2300m), Gangtok – Pakyong - Rhenok (1650-1500-1000m). etc. (Plate 2.1.shows some of the locations of the study area).

2.1.1. COLLECTION AND PRESERVATION OF MATERIALS

During the field trips the different species of *Acer* along with other plant specimens relevant to the present dissertation were traced on visual observation and representative specimen were collected. The specimen included leaves, flowers or fruits. In case the flowers and fruits were not available, specimen with healthy and mature leaves were collected. During the winter season the winter twigs with winter buds were collected. The specimen were tagged and recorded in the Field Note Book and temporarily pressed in portable herbarium press between blotting papers. On return to the laboratory the specimens were cleaned trimmed and transferred to wooden plant press for drying after poisoning with 4% Mercuric Chloride solution in rectified alcohol. After proper drying the specimens were poisoned by soaking with 4% Mercuric Chloride solution in rectified sprit, dried again under pressure using blotting papers.

Dried and poisoned specimen were then pasted on standard herbarium sheets, labeled properly and temporarily stored in the Herbarium cabinet in the Plant Taxonomy Laboratory, North Bengal University.

2.1.2. FIELD NOTE BOOK

All the collected specimens were recorded in a Field Note Book which included the specific locations, altitude, date of collections, availability, habit, habitat, flower colour such characters of plants which are not available with dry herbarium specimens. The field notes were transferred to herbarium labels for ready reference. After conclusion the Note Book will be deposited at the NBU- Herbarium.

2.1.3. IDENTIFICATION

The specimens were primarily identified in the Plant Taxonomy Laboratory of North Bengal University, using the available literature and matching with the available predetermined specimens. Identification was confirmed by matching all specimen at the central National Herbarium, Howrah (Calcutta), the herbarium of the Llyod Botanical Garden, Darjiling and at the herbarium of Sikkim Himalayan Circle of Botanical Survey of India (BSHC).

2.1.4. ENNUMERATION AND DESCRIPTION

The enumerations of the different species of Acer have been in an alphabetical manner for easy handling. For each species the correct name followed by basionym and other available synonyms has been provided. Proper author citation, references to protologue and record in floristic and taxonomic work as correct name has been provided with the key to the acronym and abbreviation used to denote various journals and books being given at the beginning of the enumeration. This is followed by the local name(s) available with the language of the name being indicated. For flowering and fruit maturity a month, to month range has been provided. The different species growing over a wide altitudinal range tends to show variation in flowering time. In such cases a total range have been provided.

Detailed description of each species has been provided with all the measurements being provided in the metric system only. In case of the measurements if not specifically mentioned, the length of the structure is placed first followed by the breadth punctuated by `x' mark e.g. (4x3 cm denotes that the length = 4cm and the breadth 3 cm.)

To cite the voucher specimen, the place of collection followed by altitude of the place, date of collection, the collector's names and field numbers have been provided with the specimens from different places of collections being provided separately. Local distribution provided in the enumeration are based both on collected specimens and observation made during the field trips with no actual collection and is based upon the observation and collection of the present work not based upon any literature. On the other hand, information related to the general distribution have been determined from past literature and deposited specimen from the different herbaria visited. At the end of the enumeration a `NOTE' has been provided which covers (i) the local uses of plants in any (ii) cytological information (iii) nature of the bark and any other observations of interest made during the field study. Photographs on habitat, habit has been provided along with line drawings.

2.2. CYTOLOGICAL INFORMATION

The diploid (2n) chromosome number of the different species have been recorded from available literature are cited in the enumeration at the end of the morphological description under the Note. The chromosome number were collected from the *Chromosome Atlas of Flowering Plants of the Indian Subcontinent* (Kumar and Subramaniam 1986) and *Maples of the World* (D. M. van Gelderen, P.C. de Jong and H. J. Oterdoom, 1994).

2.3. PHYTOSOCIOLOGICAL STUDIES

Phytosociological studies were carried out with a primary aim to determine the status of availability, horizontal and altitudinal distribution and to know the natural associates of the different species of *Acer.*

2.3.1. LOCATIONS OF THE STUDY AREA

The study area included all those locations described above in 2.1 where the different species of *Acer* were found to occur. For better understanding the different localities which were visited were divided into four altitudinal zones (i) 800-1600m (ii) 1600-2400 m (iii) 2400-3200 m and (iv) 3200 and above.

2.3.2. METHODS FOR SAMPLING

Trees and tree saplings above 30 cm in height were classified as trees during the count. The tree and shrub layers were analyzed by quardat method using the sizes 10 x 10 m and 5 x 5 m respectively. A total of 40 sites (with a total of 160 sites) were take at each altitudinal zone with the 5 x 5 m quadrate being inside the 20 x 20 m quadrat (P. S. Yadav *et al*, 1991, S. Nath *et al* 1991). The sites selected were random except that each 20 x 20 m quadrats were so chosen that it contained at least one or more species of *Acer.* Specimens of the different species were collected tagged and herbarium prepared for identification.

2.3.3. PROCESSING OF THE DATA

The vegetational data were quantitatively analyzed for Relative frequency, Relative Density following Curtis and McIntosh (1950), which were calculated as follows.

1. **Relative Density (RD)** =

$$\frac{\text{Number of the individuals of the species}}{\text{Number of individuals of all the plant species}} \times 100$$

2. **Relative Frequency (RF)** =

$$\frac{\text{Number of points of occurrence of the species}}{\text{Number of points of occurrence of all the plants}} \times 100$$

As it was not possible to calculate the Relative Dominance (RD) due to the lack of sufficient data the simplified calculation of the Importance Value (IV) was carried out following Das and Lahiri (1997) where the Importance Value was calculated as the sum of the Relative Density and Relative Frequency. As the data was not fully sufficient only a list count of the various associated plants could be tabulated.

2.4. PHENOLOGICAL STUDIES

The phenological observations were made on the four phenophases i.e., leaf and or inflorescence sprouting; flowering and anthesis; fruit setting, development and retention and leaf and fruit drop, during two successive years from February 1997- February 1999. The phenological observations were made following Lawerence(1955); Oper *et al.*(1980), Ralhan *et al.* (1985) and Ralhan *et al.* (1991). For most of the species two point of growth were established at two sites representing the lower and the upper limits of the growth of the species within the micro catchments. This was carried out to establish the whole array of variation in the temporal differentiation among the phenophase due to the micro-climatic conditions. Repeated visits were made to the field during the period and a number of randomly chosen individuals were studied for detailed data recording. A particular phenophase was considered to have started when about 10% (Semalty and Sharma 1996) were observed in the phase and the phase was considered completed when only less than 10 % of the individuals remained in that particular phase.

2.4.1. STUDY OF FRUIT SETTING AND MATURITY

For the fruit setting studies on five trees with two branches each were tagged and observations recorded after 20 days and 50 days of inflorescence emergence. The fruit setting and retention were calculated using the formula:

$$\textbf{Fruit Setting} = \frac{\text{Total number of flowers per inflorescence - number of fruits set after 20 days}}{\text{Total number of flowers per inflorescence}} \times 100$$

$$\textbf{Fruit Retention} = \frac{\text{Number of fruits set after 20 days - Number of fruits after 50 days}}{\text{Number of fruits set after 20 days}} \times 100$$

2.5. FRUIT, SEEDS, SEEDLING AND PROPAGATION

2.5.1. STUDY OF FRUIT AND SEEDS

Morphometric studies of the fruits and seeds were made from the seeds and fruits collected from the different locations for collections with the help of a dissecting microscope. For determining the angle of wing divergence the method adopted by Banerji *et al* (1972) was followed.

2.5.2. STUDY OF SEEDLING AND SEEDLING MORPHOLOGY

Seeds collected were germinated in laboratory conditions. Seeds of the different species were separately removed from the fruits (this was done as in a number of species parthenocarpic fruits which were empty and could not be distinguished from the outside were encountered) and the healthy seeds chosen. The surface of these seeds were sterilized with 0.1% mercuric Chloride solution for three minutes and transferred to 1% silver nitrate solution to remove the mercuric chloride and washed several times in sterile distilled water. 10 healthy seeds of each species were taken in triplicates in a sterile 15 cm petri plate lined with a single layer of Whatman filter paper. Sufficient sterile distilled water about 15ml were added and placed randomly under a constant temperature of 18°C.

Seedlings were also raised in earthen pots containing sand with 15 seeds placed in a 8 cm wide pots. The seedlings that emerged were removed at different stages of development and herbarium sheets prepared after description. For calculating the germination percentage was calculated utilizing the formula given below.

Germination Percentage : The germination percentage was calculated by utilizing the formula

$$\text{Germination Percentage} = \frac{\text{Total number of Germinated seeds}}{\text{Total number of seeds sown}} \times 100$$

2.5.3. STUDY OF VEGETATIVE PROPOGATION

10 hard and soft wood cuttings were made for the different species with the lower end cut in an angle comprising of 2-3 nodes were end dipped in the commercially available rooting hormone Ceradex and planted in flat pots having a diameter of 10 inches and depth of 4 inches chiefly containing pre-sterilized sand and humus mixture in a ratio of 4:1. Triplicates for each type was taken. The pots were kept in moist condition by appropriate watering. From time to time cuttings were gently removed to determine if there was any rooting.

2.6. STUDY OF LEAF ARCHITECTURE

For the leaf morphology the both fresh as well as leaves from pressed plants were utilized. Drawings of the outline of the leaves were traced on a sheet of paper along with the major veins visible on the lower epidermis. Surface structures were studied under the simple microscope

and different magnifications of the compound light microscope and descriptions made.

2.6.1. CLEARING OF THE LEAVES

For the study of the minor venation pattern segments whole leaves were decolourized utilizing one of the techniques described below depending upon the thickness as well as the behaviour of the leaves to the treatment. In the case of the thin leaves, the leaves were directly decolourized by placing them in 5% solution of Sodium Hypochloride solution in covered petriplates (Mujica & Cutler, 1974). After complete decolourization the leaves were thoroughly washed with distilled water to remove the hypochloride solution and then passed through an alcohol grade and stained with 2 % saffranin prepared in 70% alcohol. The excess stain was washed with 70 % alcohol. After complete dehydration in absolute alcohol the stained leaves were passed through different grades of alcohol xylene mixture and finally transferred to pure xylene. It was then mounted between two glass plates in DPX and kept in an oven at 50°C to dry.

Thick leaves were cleared by placing them in 5% NaOH solution for 24 to 48 hours, till complete clearing were obtained (Foster 1949,1952, Markey 1949, Brady *et al.* 1965). It was then washed a number of times with distilled water to remove the Sodium hydroxide. In case the leaves remained opaque after clearing it was boiled in lactic acid to achieve clearing (Sands, 1973) washed a couple of times in distilled water and passed through the alcohol grades and stained as mentioned above

2.6.2. STUDY OF MINOR VENATION

The stained segments were observed under 5x objective of a compound microscope using a 5x eye piece. Camera lucida drawings were then made from the median portion that included the mid vein along with the secondary veins. The venation at the marginal portion was also drawn in a similar manner. Descriptions were made following the scheme of Hickey 1973.

2.6.3. STUDY OF VEIN ISLET NUMBER

Utilizing the stage micrometer and the camera lucida a $4mm^2$ square was drawn on a sheet of paper onto which the camera lucida drawing of the veins were made. For each species 30 such drawings comprising of 10 from the median portion, 10 from the apical portion and 10 from the basal portions were made. The vein islet number were then determined by counting the islets

and dividing by 4 in each case to determine the number per mm^2. While those incomplete islets on the left and the top were not considered those to the right and the bottom were counted. The average number was then calculated and tabulated.

2.6.4. STUDY OF APPENDAGES

For the determination of the location and the type of appendages the decoluorized leaves were observed under the high power objectives of the compound light microscope. The types of the hairs and their distribution along with their measurements were made utilizing the occular micrometer after proper standardization of the microscope.

2.6.5. STUDY OF STOMATA

From leaf peelings of the lower epidermis camera lucida drawing were made and measurements of the stomata and guard cells were carried out with the help of the standardized ocular micrometer. The diameter of the field of the microscope under the high power using a 10 x eyepiece was determined with the help of the stage micrometer. The number of stomata in a field was counted. 75 such readings for each species taking 25 readings from the basal portion of the leaf, 25 from the median and 25 from the apical portion were taken and the Stomatal Frequency and Stomatal Index were determined by using the formula

$$\text{Stomatal Frequency} = \frac{\text{Total number of stomata in each field}}{\text{Area of the field in mm}}$$

$$\text{Stomatal Index} = \frac{\text{Total number of stomata in each field}}{\text{Number of stomata + Number of Epidermal cells in the field}} \times 100$$

2.7. STUDIES ON POLLEN MORPHOLOGY

The external morphology of the pollen grains of the available species of *Acer* were studied. Anthers from flowers and / or mature buds (just prior to blooming) were collected dried and preserved in a desiccators with silica gel. The acetolysis methods of method of Erdtman (1960) with modifications of Chanda (1966) and Nair (1970) were followed for the study.

2.7.1.1. PREPARATION OF ACETOLYSIS MIXTURE

The acetolysis mixture was prepared by slowly adding one part concentrated Sulphuric acid to

nine parts of Acetic anhydrite. Each time freshly prepared acetolysis mixture was used.

2.7.1.2. DETAILS OF THE ACETOLYSIS METHOD FOLLOWED

1. Fresh or Dry polliniferous material were crushed over a finely meshed pre-flamed (to avoid species to species contamination) stainless steel sieve (0.11 sq mm) resting on a funnel; setting on a hard glass centrifuge.
2. After proper crushing 10 ml of acetolysis mixture was slowly poured over it and stirred with a clean dry glass rod.
3. The centrifuge tube containing the mixture were heated in a water bath or kept in an oven at 60 ° until the mixture turned brown.
4. The tubes were allowed to cool and then centrifuged at 4,000 r.p.m. for 5 minutes and decanted.
5. The sediment was then washed by adding a small of distilled water shaken thoroughly, and then centrifuged 4,000 r.p.m. for 5 minutes and then throwing off the water.
6. This process was repeated 3-4 times to completely remove the acetolysis mixture.
7. 10 ml of distilled water was then added and the mixture thoroughly shaken and distributed equally in two tube. While one tube was set aside for chlorination to the other 2ml Of 50% glycerine was added after centrifugation and decantation.
8. The other tube was subjected to chlorination by adding two drops of concentrated hydrochloric acid followed by few drops of 4% sodium hypochloride solution and stirring it with a clean glass rod.
9. The washing as described above in step 6 was followed to remove all the chemicals.
10. The content of the second tube was then mixed with the other kept in glycerine and was washed once again.
11. A drop of 50% glycerine was then added to the sediment and the tubes were kept in a inverted position over a piece of blotting paper over night to allow the drainage of excess water.

2.7.1..3. PREPARATION OF THE POLLEN SLIDES

The process as described by Kisser (1935) was followed for the preparation of the slides.

1. A very small amount of glycerine jelly was taken at the tip of a platinum needle that was then inserted into the tube containing the pollen bearing sediment and the surface was touched. The jelly was then transferred onto a clean glass slide and covered with a 18 mm round or square cover glass.
2. A few chips of wax (melting point 60°-62°) was placed on one margin of the cover glass

And the slide gently heated below the jelly to spread the jelly along with the specimen and at the same time to allow entry of molten wax for sealing.

3. The slides were kept on a flat horizontal surface and allowed to cool.
4. The excess was from the surface were cleaned with the help of a clean soft cloth. The slides were labeled with reference to the specimen, date of preparation and utilized for further palynological studies.

2.8. STUDY OF ALLELOPATHY

To determine the alleopathic effets seven species of *Acer* seven belonging to five series and placed under five sections according to Oterdoom (1994) were selected . The effect of their leaf extracts and leaf leachetes were tested against six plants belonging to different families. Three cultivated plants *Pea, Maize* and *Cauliflower* and two wild herbs *Urtica dioeca* and *Galliumsoga parviflora* were used for this purpose. Mature seeds of the wild species were collected from the fields and stored at 4° C in brown paper envelopes. Seeds of the cultivated plants were purchased from the horticulture stores. Branches with leaves of the different species of *Acer* collected in the fields in polythene bags were immediately stored in ice boxes were brought to the laboratory from the field. The procedures described by Datta and Ghosh (1987) was followed for this work.

2.8. 1. PREPARATION OF EXTRACT AND LEACHETE SOLUTIONS

After thoroughly washing the leaves in distilled water to remove any adhering particles the leachetes was extracted by soaking 100 g of fresh materials in distilled water for 72 hrs. The leachetes were then filtered through Whatman (N01) filter paper and the volume made upto 250 ml by adding distilled water. This was used as the stock solution which was expressed by its ratio as 1:2.5. The stock solution of the leaf extract 1:2.5 was prepared by taking thoroughly washed fresh leaves but the extraction was carried out after grinding it into a paste in a Bajaj Mixing Machine. The desired concentration of 1:5,1:10,1:20 of both the leachetes and extracts were prepared consequently from the stock by subsequent dilution with distilled water.

2.8.2. GERMINATION TESTS

Seeds of test plants were surface sterilized with 0.1% mercuric Chloride solution for three minutes and transferred to 1% silver nitrate solution to remove the mercuric chloride and washed several times in sterile distilled water. 30 healthy seeds in case of Cauliflower, *Urtica dioeca*, *Galliumsoga parviflora* and Cauliflower and washed thrice with distilled water, 15

healthy seeds of *Pisum sativum* and 10 *Zea mays* were placed in a sterile 15cm glass petriplates lined with single layer of Whatman filter paper. The filter paper was moistened sufficiently by adding 15ml of the test solutions. Replicas in triplicates for each test solution were made along with a control with 15 ml of sterile distilled water. The petriplates were randomly placed and under a constant temperature of 18° C. Emergence of the lateral roots were taken as the criterion of seed germination. The linear length of the primary roots, shoots and the seedlings were made after 14 days. In case of *Zea mays, Pisum sativum* and cauliflower the length of other adventitious roots were and their number counted. In the others the number and the length of the lateral roots were counted and measured.

The data thus obtained for the different parameters of seed germination and seeding growth were statistically analyzed and presented in tabular forms. The different parameters of seed germination and growth analyzed are given below.

2.8.2.1. GERMINATION PERCENTAGE :The germination percentage was calculated by utilizing the formula

$$\text{Germination Percentage} = \frac{\text{Total number of Germinated seeds}}{\text{Total number of seeds sown}} \times 100$$

2.8.2.2. GERNIMATION PERCENTAGE INHIBITION OR STIMULATION : The formula of Saxena *et. al,* (1995) was used for the calculation of the Germination percentage inhibition or stimulation

$$\text{Percentage inhibition or stimulation} = \frac{\text{Germination \% in desired solution} - \text{Germination \% in control}}{\text{Germination \% in control}} \times 100$$

2.8.2.3. PERCENTAGE OF VIABILITY : Percentage of viability was calculated by using the formula according to Acharyya (1998)

$$\text{Percentage viability} = \frac{\text{Total number of viable seeds in the desired solution}}{\text{Total number viable of seeds in the Control}} \times 100$$

2.8.2.4. PERCENTAGE OF NON-VIABILITY: Percentage of nonviable seed was calculated by using the following formula

$$\text{Percentage viability} = \frac{\text{Total number of non-viable seeds in the desired solution}}{\text{Total number non-viable of seeds in the Control}} \times 100$$

2.8.2.5. PERCENTAGE INHIBITION OF SEEDLING LENGTH: Percentage of inhibition or

stimulation of seedling length was calculated using the formula

$$\text{\% Inhibition of Seedling length} = \frac{\text{Length of seedling in desired solution} - \text{length of seedling in Control}}{\text{Length of seedling in the Control}} \times 100$$

2.8.2.6. PERCENTAGE INHIBITION OF ROOT LENGTH : Percentage of inhibition or stimulation of root length was calculated using the formula

$$\text{\% inhibition of Root length} = \frac{\text{Length of root in desired solution} - \text{length of root in Control}}{\text{Length of root in the Control}} \times 100$$

2.8.2.7. PERCENTAGE INHIBITION OF SHOOT LENGTH : Percentage of inhibition or stimulation of shoot length was calculated using the formula

$$\text{\% inhibition of Shoot length} = \frac{\text{Length of shoot in desired solution} - \text{length of shoot in Control}}{\text{Length of shoot in the Control}} \times 100$$

2.8.2.8. SHOOT VIGOUR INDEX: Shoot Vigour Index was calculated following the formula described by Thind and Malik(1988)

$$\text{Shoot Vigour Index} = \text{Germination \%} \times \text{Shoot Length}$$

2.8.2.9. ROOT VIGOUR INDEX: Root vigour index was calculated by using the following formula

$$\text{Root Vigour Index} = \text{Germination \%} \times \text{Root Length}$$

2.8.2.10. SEEDLING VIGOUR INDEX: For the calculation of the Seedling Vigour index the following formula was adopted.

$$\text{Seeding Vigour Index} = \text{Germination \%} \times \text{Seeding Length}$$

2.8.2.11. SHOOT ROOT RATIO : The formula of Bajpai *et al* (1995) was used for the calculation of the shoot :root ratio.

$$\text{Shoot : Root Ratio} = \frac{\text{Length of the Shoot}}{\text{Length of the Root}}$$

2.9. STUDY OF WINTER BUDS

The resting dormant buds were collected during the months of January-February when the trees were leafless except for *A.oblongum* Wall. ex DC. which is evergreen and *A.thomsonii* (Miquel)Murray whose buds were collected during October-November after leaf fall. The twigs with the buds were collected in polythene bags that were properly labelled. These were brought to the laboratory and studies made after dissection under the dissecting microscope.