HOW TO WORK

WITH

THE MICROSCOPE.

A Course of Lectures

ON

THE PRACTICAL USE OF THE INSTRUMENT AND MICROSCOPICAL MANIPULATION,

BY

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Illustrated Edition with 32 Plates, containing upwards of 150 Figures.



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The Author reserves the right of translating this Work.

DEDICATED

TO MY FRIEND

FERGUSON BRANSON.

PREFACE

TO THE

ILLUSTRATED EDITION.

With the view of increasing the usefulness of this work, numerous explanatory illustrations have been added in the present edition.

The author, both in the text and in the explanations to the engravings, has endeavoured to restrict himself, as far as possible, to giving hints and directions which may be practically useful to the student while he is at work. All matter that would be merely interesting to the general reader has therefore been altogether omitted. Directions for working cannot well be too explicit and precise, and the more simply they are given, the more useful they are likely to be.

The structure of the microscope, its history, the principles of its action and the appearance of various objects examined in it, are fully discussed in the larger works of Professor Quekett, Dr. Carpenter, Mr. Hogg, and others enumerated in the note on page 4, to which the reader is referred.

The Author trusts that the Student will carefully go through the tables commencing on page 104, as he feels satisfied that any one who will patiently submit to the routine and trouble, will be amply rewarded by the practical knowledge he will thus gain of the use of the instrument.

L. S. B.

61, *Grosvenor Street, January*, 1861.

PREFACE

TO THE

FIRST EDITION.

An earnest desire to assist in diffusing a love for microscopical inquiry, not less for the pleasure it affords to the student, than from a conviction of its real utility and increasing practical value in promoting advancement in various branches of art, science, and manufacture,—a wish to simplify as far as possible the processes for preparing microscopical specimens, and the methods for demonstrating the anatomy of different textures,—and the belief that many who possess microscopes are deterred from attempting any branch of original investigation solely by the great difficulty they experience in surmounting elementary detail and mere mechanical operations,—are my chief reasons for publishing this elementary course of lectures, which was delivered during the past winter.

It has been thought desirable to append the tables which I have been accustomed to use in my course of practical demonstrations, for the purpose of enabling everyone to practise for himself the most useful branches of manipulation. Each table will occupy the student about two hours.

Subjoined is a list of the apparatus required for microscopical research, much of which is simple and inexpensive. A number has been added to each instrument, by transmitting which to any instrument-maker, the observer will be furnished with the apparatus required.

L. S. B.

Pathological Laboratory,
Carey Street, Lincolns-inn, June, 1857.

DESCRIPTION OF THE SIMPLE FORM OF POCKET MICROSCOPE FIGURED IN THE FRONTISPIECE.

This instrument was designed for clinical investigation, but it is adapted for field work and for mieroscopieal inquiries generally.

Fig. 1. The mieroseope, half the real size. It eonsists of thin tubes, *a*, *b*, and *c*; *a* earries the eye-piece, is four and a-half inches long, and slides in *b*, which is the same length, but only slides up to its eentre in the outer tube *c*. Tube *b* carries the objeet-glass, * points to a bolt whieh ean be fixed by aid of a raek and tooth, at any height, aeeording to the foeal length of the object-glass. This arrangement prevents the risk of the objeet-glass being foreed through the preparation while foeussing. At the lower part of the body are seen *d*, the serew for fixing the preparation in any particular position; *e* the aperture for throwing the light on opaque objects; and *f*, part of the spring whieh keeps the preparation in contaet with the flat surfaec below, while the requisite movements are made with the hand.

Fig. 2. Shows the strueture of an animalcule eage for examining infusoria, parts of plants, or any moist preparations with this instrument.

Fig. 3. Is a little screw with an arm for fixing the specimen in any particular position that may be desired. Its arrangement is shown in figs. 1 and 5, *d*.

Fig. 4. Mirror employed for examining objects by transmitted light. It may be placed on the table, and can be inclined at any angle.

Fig. 5. Under surface of the body or stage, showing the flat rim (*g*) upon which the slide is placed; *h* the under surface of the object-glass, *f* the spring.

Fig. 6. The stage, which unscrews from the lower part of the body. The shape of the spring (*f*) is represented.

In using the instrument, the slide with the object to be examined is placed upon the stage, the thin glass being upwards towards the object-glass, while the spring is made to press upon the *under* surface of the slide. The little screw (*d*) is removed. The slide may now be moved in every position, and any particular object to be examined can readily be placed exactly under the object-glass. Tube *a* is withdrawn about two-thirds of its length. The tube *c* being firmly held in the left hand, *b* is grasped with the right, and with a screwing motion the object-glass is brought to the right focus; the little bolt is moved up to its proper place, and the object may be examined either by holding the instrument up to the window or by causing the light to be reflected from the mirror. Focussing different parts of the object is easily effected by moving the tube *a*, which is made to *slide* up and down more easily than *b*.

When this instrument is required for class purposes, the preparation is fixed in position by the screw *d*, and it may then be passed round without fear of displacement. Any powers may be used from the twelfth to the two-inch.

The microscope may be mounted on a stand like a telescope.

In examining objects by reflected light, an ordinary candle is alone required. A bull's-eye condenser is not necessary.*

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* The instrument is made by Mr. Matthews, Portugal Street, Lincoln's Inn Fields. The price, with eye-piece, is 25*s*. Any object-glass can be adapted.

CONTENTS.

LECTURE I.

PAGE

Introduction.—The simple and compound microscope.—Optical portion of the microscope.—The eye-piece.—The object-glasses.—Spherical and chromatic aberration.—Angle of aperture.—The mirror.—Mechanical portion of the microscope.—Adjustments for altering the focus.—The body of the microscope.—Diaphragm.—Microscope makers.—Students' microscopes.—Necessary apparatus.—Binocular microscope.—Travelling and dissecting microscopes.—Large microscopes 1

LECTURE II.

Examination of objects by reflected light, transmitted light, and polarised light.—*Reflected light.*—Different methods employed.—By bull's eye condenser or side reflector.—By Lieberkuhn.—Dark ground illumination.—Parabolic reflector.—Annular condenser.—*Transmitted light.*—Diaphragm.—Achromatic condenser.—Gillett's condenser.—*Polarized light.*—Polarizer.—Analyzer.—Iceland spar.—Tourma line.—Iodo-quinine or Herapathite.—*Illumination by artificial light.*—Lamps.—Camphine lamp.—Oil lamps. Argand lamp.—French moderator lamps.—Gas lamps.—Of the importance of protecting the eyes from the diffused light of lamps.—*On drawing microscopical specimens.*—Camera lucida.—Steel disc.—Neutral tint glass reflector.—*On measuring objects.*—Cobweb micrometer.—Stage micrometer.—Directions for measuring objects.—Measuring the angles of crystals.—On ascertaining the magnifying power of object glasses . . . 13

LECTURE III.

PAGE

Instruments required in microscopical research.—Spirit lamp.—Wire retort stands.—Water bath.—*Instruments for cutting thin sections of tissues.*—Scalpels.—Double-edged Scalpel.—Double-bladed or Valentin's knife.—Scissars.—Needles.—Forceps.—*Apparatus used for examining objects in the microscope.*—Glass slides.—Thin glass.—Glass cells.—Watch glasses.—*Cements.*—Gold size.—Shell lac.—Brunswick black.—Marine glue.—Cement for attaching India-rubber or gutta percha to glass slides.—Canada balsam.—Gum.—French cement composed of lime and India-rubber.—*Preservative solutions.*—Spirit and water.—Glycerine.—Thwaites' fluid.—Solution of naphtha and creosote.—Solution of naphtha and water.—Solution of chromic acid.—Preservative gelatine.—Gelatine and glycerine.—Solution of chloride of calcium.—Arseniuretted hydrogen.—Nitrogen 25

LECTURE IV.

On the methods of making cells for microscopical preparations.—Cells for preserving microscopial specimens.—Cells for dry objects.—Brunswick black cell.—Cells made of tinfoil and marine glue,—Of cutting and grinding glass—Of cutting the thin glass.—Stone or pewter slab for grinding glass.—Cementing glass together with marine glue.—Cleaning off superfluous glue.—Cells made of thin glass.—Simple methods of perforating the thin glass.—Deeper glass cells.—Small deep glass cells for injections.——Built glass cells.—Deep glass cells made by bending a strip of glass in the blowpipe flame.—Moulded glass cells.—Troughs for examining zoophytes.—Animalcule cage.—Round cells proposed by Dr Guy . . 40

LECTURE V.

On examining objects in the microscope.—*General considerations upon the structure of tissues.*—Flesh or muscular tissues.—On demonstrating the anatomical peculiarities of tissues.—The anatomy of organs more easily demonstrated in the lower animals than in man and the higher animals.—Of the time after death when tissues should be examined.

PAGE

—Ciliary motion.—*Of preparing tissues for microscopical examination.*—Of making minute dissections.—Dissecting under the surface of fluid.—Tablets upon which dissections may be pinned out.—Of obtaining thin sections of different textures for microscopical examination.—Drying the tissue before cutting the section.—Hardening the tissue.—Horn.—Hair.—Making thin sections of bone.—Teeth.—Sections of wood.—Of dissecting tissues under the microscope with the aid of the compressorium.—*Of the importance of examining objects in various ways.*—Appearances of the same object in air, water, and Canada balsam by transmitted light, and under the influence of reflected light and polarized light 47

LECTURE VI.

On examination of tissues and their preservation as permanent objects.—General consideration with reference to the nature of the medium in which tissues should be placed for examination.—*Examination and preservation of structures in air.—Examination and preservation of substances in aqueous fluids.—Examination and preservation of soft tissues;* muscle.—Arrangement for pressing down the thin glass cover upon the preparation while the Brunswick black is drying.—Examination of vegetable tissues.—Of the circulation in the cells of vallisneria.—*Examination and preservation of objects in Canada balsam.*—Examination of hard tissues; bone.—*Air bubbles.—Oil-globules.—Of the separation of deposits from fluids.*—The pipette.—Removing the deposit with the pipette.—Examination of infusoria.—Vorticellæ.—Zoophytes.—On separating the course from the finer particles of a deposit.—Method of obtaining the silicious skeletons of lower organisms.—Wash bottle.—Of keeping preparations in the cabinet 59

LECTURE VII.

Of injecting.—Natural and artificial injections.—Transparent and opaque injections.—Instruments required for making injections.—Syringe, pipes, corks, bull's-nose forceps, needle for passing the thread round the vessel.—*Of opaque*

PAGE

injections.—Injecting cans.—Size.—Colouring matters.—Vermilion.—Chromate of lead.—White lead.—*Of transparent injections.*—Injecting with plain size.—Colouring matters.—Carmine.—Prussian blue.—Advantages of employing Prussian blue.—Composition of the Prussian blue fluid for making transparent injections.—Mercurial injections.—*Injecting the lower animals.*—Mollusca.—Insects.—Of the practical operation of injection.—Of injecting the ducts and secreting follicles of glands.—Of preparing portions of injected preparations for microscopical examination 72

LECTURE VIII.

Of the advantages of chemical reagents in microscopical investigations.—Instances of the use of reagents.—*Of the hardening properties of different solutions.*—Mr. Lockhart Clarke's plan of preparing thin sections of the cord.—Method of rendering tissues hard and transparent.—Chemical reagents required by the microscopist.—Testing for carbonate and phosphate of lime, phosphate of ammonia and magnesia, sulphates, and chloroides.—Of taking notes of microscopical observations.—Of the importance of making sketches.—Of taking photographs of objects in the microscope.—Of making observations upon objects in the microscope.—Of drawing inferences from observations.—*Fallacies to be guarded against in microscopical investigation.* Errors of observation.—Of the commencement and termination of tubes.—On the difficulty of seeing structures from their transparency.—Fibres and membranes artificially produced by actions of reagents.—A fibrous appearance produced in structureless membrane.—Collection of oil-globules appearing as if within a cell-wall.—On the accidental presence of extraneous substances.—Conclusion . 85

TABLES FOR PRACTISING THE USE OF THE MICROSCOPE AND MANIPULATION 104

APPARATUS REQUIRED IN MICROSCOPICAL INVESTIGATION . 112

LIST OF ILLUSTRATIONS.

FRONTISPIECE.—SIMPLE FORM OF COMPOUND MICROSCOPE ARRANGED AS TELESCOPE.

PLATE I. STRUCTURE OF SIMPLE AND COMPOUND MICROSCOPE—EYE-PIECE, AND OBJECT GLASS.

PLATE II. ANGLE OF APERTURE—SPHERICAL AND CHROMATIC ABERRATION —STAGE AND MIRROR OF MICROSCOPE.

PLATE III. STUDENT'S MICROSCOPE.

PLATE IV. BINOCULAR MICROSCOPES—MICROSCOPE FOR TWO OBSERVERS.

PLATE V. TRAVELLING MICROSCOPE, ARRANGED UPRIGHT AND IN A SLANTING DIRECTION.

PLATE VI. TRAVELLING MICROSCOPE, ARRANGED FOR EXAMINING OBJECTS IN A VIVARIUM AND FOR MAKING DISSECTIONS.

PLATE VII. BULL'S-EYE CONDENSER—LIEBERKUHN AND STAGE FORCEPS.

PLATE VIII. MICROSCOPE ARRANGED FOR EXAMINING OBJECTS BY REFLECTED AND BY TRANSMITTED LIGHT.

PLATE IX. POLARIZING APPARATUS — GAS AND CAMPHINE MICROSCOPE LAMPS.

PLATE X. ARRANGEMENT AND INSTRUMENTS FOR DRAWING OBJECTS— MICROMETER LINES FOR MEASURING OBJECTS.

PLATE XI. GONIOMETER FOR MEASURING THE ANGLES OF CRYSTALS — OCTOHEDRAL AND RHOMBOIDAL CRYSTALS—ACHROMATIC CONDENSER.

PLATE XII. LAMPS AND SUPPORTS — BRASS PLATE — TRIPODS — WATER BATHS.

PLATE XIII. SCISSARS — SPRING SCISSARS — SECTION KNIFE — DOUBLE-BLADED OR VALENTIN'S KNIFE—FORCEPS.

PLATE XIV. INSTRUMENTS FOR MAKING GLASS CELLS AND CUTTING CIRCLES OF THIN GLASS—WRITING AND CUTTING DIAMOND.

PLATE XV. MAKING SHALLOW AND DEEP GLASS CELLS.

PLATE XVI. BUILT GLASS CELLS FOR THICK PREPARATIONS.

PLATE XVII. FATTY TISSUE—CAPILLARY VESSELS.

PLATE XVIII. WHITE AND YELLOW FIBROUS TISSUE—TRACHEÆ FROM AN INSECT—ADIPOSE TISSUE—INJECTED VESSELS.

PLATE XIX. INSTRUMENTS FOR DISSECTING UNDER WATER, AND FOR CUTTING THIN SECTIONS OF WOOD, BONE, &c.—COMPRESSORIUM.

PLATE XX. DIFFERENT APPEARANCES OF THE SAME OBJECTS EXAMINED BY REFLECTED, TRANSMITTED, AND POLARIZED LIGHT.

PLATE XXI. APPARATUS FOR EXAMINING OBJECTS IN FLUID, AND FOR SEPARATING DEPOSITS FROM FLUIDS.

PLATE. XXII. VESSELS FOR PRESERVING CEMENTS — GLASS SHADES AND AIR-PUMP.

PLATE XXIII. AIR BUBBLES—OIL GLOBULES, FREE AND ENCLOSED IN CELLS —BLOOD CORPUSCLES—MARGARIC AND STEARIC ACIDS—MARGARINE.

PLATE XXIV. APPARATUS FOR SEPARATING DEPOSITS FROM FLUIDS.

PLATE XXV. CRYSTALS OF CREATINE.—MICROSCOPIC FUNGI—ENTOZOA FROM THE FOLICLES OF THE SKIN.

PLATE XXVI. INSTRUMENTS AND APPARATUS REQUIRED FOR MAKING INJECTIONS.

PLATE XXVII. SIZE OF THE PARTICLES OF DIFFERENT COLOURING MATTER USED FOR INJECTION.

PLATE XXVIII. THE MINUTE ANATOMY OF THE SPINAL CORD AS SHOWN BY MR. LOCKHART CLARK'S MODE OF PREPARATION.

PLATES XXIX. BOTTLES WITH CAPILLARY ORIFICES FOR HOLDING TEST SOLUTIONS—TEST TUBES.

PLATES XXX. INVERTED MICROSCOPE — APPARATUS FOR EXAMINING OBJECTS AT A HIGH TEMPERATURE.—PHOTOGRAPHIC CAMERA.

PLATE XXXI. EXTRANEOUS MATTERS WHICH OFTEN FALL ACCIDENTALLY INTO MICROSCOPICAL PREPARATIONS WHEN THEY ARE BEING MOUNTED.

LECTURES

ON THE

MICROSCOPE

AND

MICROSCOPICAL MANIPULATION.

LECTURE I.

Introduction.—The simple and compound microscope.—Optical portion of the microscope.—The eye-piece.—The object glasses.—Spherical and chromatic aberration.—Angle of aperture.—The mirror.—Mechanical portion of the microscope.—Adjustments for altering the focus.—The body of the microscope.—Diaphragm.—Microscope makers.—Students' microscopes.—Necessary apparatus.—Binocular microscope.—Travelling and dissecting microscopes.—Large microscopes.

Gentlemen,—The course of lectures which I am about to commence will embrace the consideration of many subjects of a strictly practical character, and it will, I fear, be found devoid of that interest which necessarily attaches itself to brilliant experiments and theoretical speculations.

My aim will be, to describe to you the mode of examining different objects in the microscope, the best methods of displaying their structure, and the manner in which they may be preserved permanently. How best to demonstrate the peculiarities of a structure is a question often asked by the microscopist, and it is an important one, for upon the method employed very much depends. The success which attends our efforts in this field of research is, I believe, in great measure dependent upon our knowledge of the various methods which experience has shown to be advantageous for

rendering the anatomical peculiarities of a texture clear and distinct.

The consideration of questions involving such practical detail as these will necessarily excite far less interest than a description of the various structures which may be submitted to microscopical examination, and the consideration of their wonderful adaptation to the purposes they were intended to serve in the economy of living beings.

The utility of microscopical manipulation, and the absolute necessity of a knowledge of it, to all who work with the microscope must be my apology, if, as is probable, you should find me tedious, and feel that I am trespassing too far upon your patience.

From my position as a teacher of physiology and morbid anatomy in a large medical school, I have been naturally led to direct my attention chiefly to those branches of microscopical investigation which belong more particularly to my own department, or which bear directly upon the investigation and treatment of disease; but in the remarks which I shall have to make I shall endeavour to exclude everything of a strictly professional character. I shall allude only to those processes applicable to general microscopical research, and to the investigation of animal and vegetable tissues.

You may, perhaps, stigmatize many little points to which I shall have to allude as merely mechanical, others may be regarded as belonging rather to the province of the chemist than to the microscopical observer, and not a few will strike you as unimportant and hardly worthy of attention.

I feel very strongly how intimately success in microscopical as well as chemical inquiries, is connected with a readiness in surmounting comparatively small difficulties and with the possession of mechanical dexterity; and I should be doing a great injustice to my pupils if I did not bring these processes under their notice, and endeavour to facilitate as far as possible the performance of those operations which are essential to the successful demonstration of the arrangement of textures under the microscope. Depend upon it, these are questions not beneath the consideration of any one who takes a real interest in the structure of the different organisms by which he is surrounded, and it is the same in this as in other branches of

inquiry, that he who is most fully conversant with elementary detail will be the most successful in the consideration of the higher and more abstruse problems, while he will feel a real love for his work, which is denied to the mere superficial inquirer.

To endeavour to discover new methods of investigation appears to me to be one of the most important duties of every observer. To communicate these to his pupils must be the desire of every teacher of any branch of natural science.

By describing the results of the investigations of others we are enabled to diffuse knowledge. By detailing the conclusions we have arrived at from our own investigations, each may contribute his mite to the gradually increasing stock of information; but in impressing strongly on his pupils the nature of the successive steps by which conclusions in scientific inquiries have been at length arrived at, and by describing to them minutely the methods employed in investigations, the teacher not only encourages his pupils to become observers and to investigate for themselves, but he places them in a position to commence new researches at a point where they have been abandoned by preceding observers.

I trust that I have said enough, not only to convince you that this branch of inquiry is of the utmost importance, and should engage special attention, but I venture to hope that these remarks will not altogether have failed in exciting in your minds that amount of interest without which my lectures would be useless and unprofitable to you.

Microscopical inquiry may be undertaken by persons in almost any position. The numerous cheap and excellent microscopes which have lately been made by many English makers have largely contributed to diffuse a knowledge of minute structure. The annually increasing sale of instruments of all classes shows how popular this branch of inquiry is becoming; yet, I fear, it must be confessed that the additions to scientific knowledge are by no means so great as a consideration of these circumstances would have led one to anticipate, and although there are many instruments, I fear it must be confessed that the observers are comparatively few.

The opinion, that it is only necessary to place an object in the field of the microscope, in order to make out its structure

seems far too prevalent. To this erroneous idea much of the disappointment suffered by many who are provided with microscopes may be traced. The difficulties which I myself have experienced at every step in original investigation have led me not to undervalue the importance of what have been termed mere mechanical operations.

I shall first of all draw your attention to the requisite qualities of a good practical instrument, and the necessary accessory apparatus. The many excellent books in our own language renders it unnecessary for me to occupy your time in a minute description of the parts of which the instrument is composed, and to these works I must refer you for information on this head.* At the same time it will be well for me to allude in general terms to certain points which every good microscope should possess, and to refer very briefly to the general form of instrument required by the student.

The Compound Microscope is the only one now used for microscopical research ; until those great improvements in the mode of making the glasses, now universally employed, had been introduced by the successful labours of Mr. Lister, Mr. Ross, and others, the compound microscope was a very imperfect instrument, and even up to the present century the simple microscope, as employed by Leeuwenhoek, and improved by Wollaston and others, possessed many advantages over its more complex but imperfect rival. I shall not attempt to explain those beautiful optical principles upon which the value of the microscope, as an instrument for minute research, depends ; these subjects are fully treated of in many of the works upon the table, and I shall therefore refer you to them for information upon these points. There are, however, several terms in constant use which I shall have occasion to allude to relating to the use of the instrument which it seems to me desirable to explain in the present lecture.

* Quekett on the Microscope.—The Microscope in Vegetable Physiology, Schacht, edited by Mr. Frederick Currey.—The Microscope and its Revelations, by Dr. Carpenter.—The Microscope, its History, Construction, and Teachings, by Mr. Jabez Hogg.—Hannover on the Microscope.—Lardner on the Microscope.—Introduction to Clinical Medicine, by Dr. Hughes Bennett.—The Microscope and its application to Clinical Medicine, by Dr. Beale.

PLATE 1.

Fig. 1.

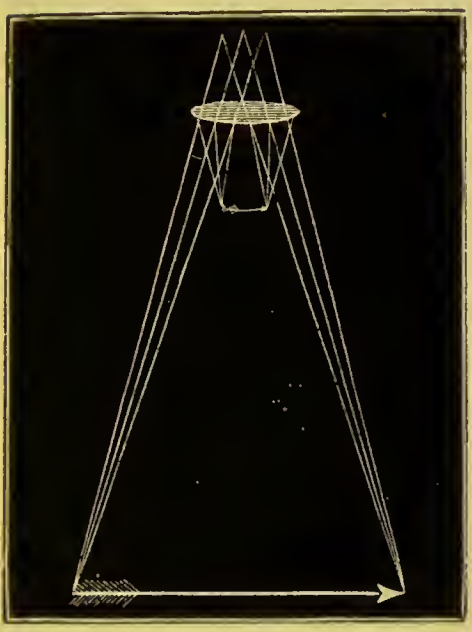


Fig. 2.

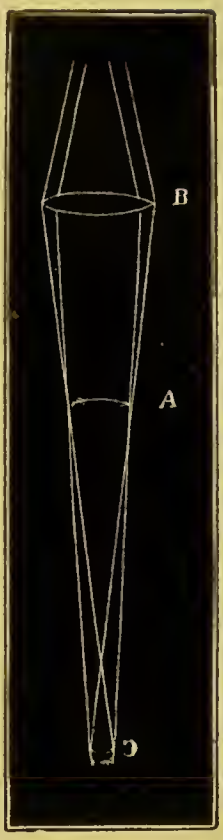


Fig. 3.

Fig. 4.

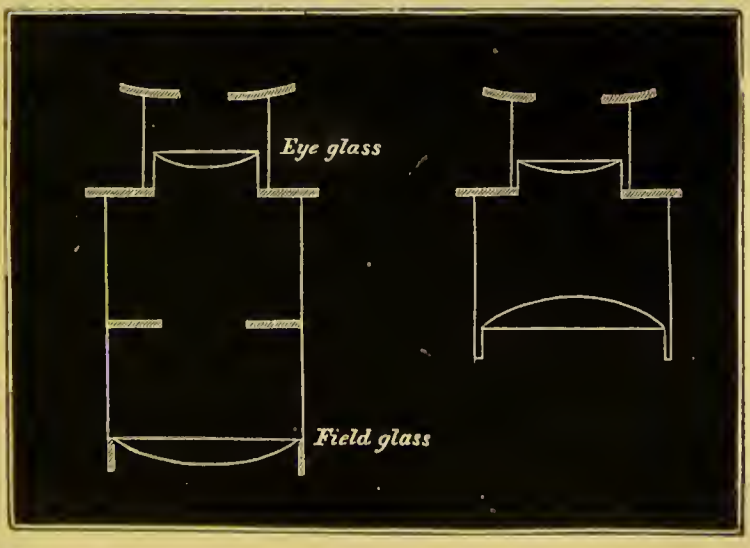


Fig. 5.



Fig. 1. Illustrates the manner in which an object is magnified by the simple microscope or by a lens (p. 5).
Fig. 2. Diagram of compound microscope. A. Point where the object is brought to a focus by the object-glass (C). The image formed at this point is magnified again by the eye-piece B (p 5).
Fig. 3. Negative or Huyghenian eye-piece. Fig. 4. Positive eye-piece invented by Ramsden (p. 5).
Fig. 5. Compound glasses of an achromatic object-glass (p. 6).

[To face page 4.]

Simple and Compound Microscopes.—In the *simple microscope* the magnified image of the object passes at once to the eye of the observer, but in the *compound microscope* the object is magnified in the first instance by the *object-glass*, and brought to a focus within the tube, as represented in this diagram. This magnified image is magnified by the *eye-piece.* The image is of course inverted, but this inconvenience may be obviated by causing it to pass through another set of lenses inserted in the tube of the microscope, and termed the *erector*. The magnifying power then, of the compound microscope may be increased either by increasing the power of the *object-glass* or that of the *eye-piece.* It must be borne in mind, however, that in increasing the power of the eye-piece, we do not magnify the object itself in a greater degree, but simply increase the size of the *image* of the object formed by the object-glass.

Any imperfections which may exist in the object-glass are thus greatly increased. Hence we should never work with deep eye-pieces, but when we wish to magnify an object to a greater degree we should adapt a higher power to the instrument.

In glancing cursorily at the structure of the microscope it will be convenient for me to allude in the first place to the *optical portion* of the instrument, and secondly to the *mechanical appliances* for moving the object, altering the focus, &c. The *optical portion*, includes the eye-piece, object-glass, and the mirror from which the light is reflected so as to pass through the object.

Optical Portion of the Microscope.

The *eye-piece* in ordinary use is the negative or Hughenian eye-piece. It consists of two plano-convex glasses, the flat surfaces of each being directed upwards. The one nearest the eye of the observer is the *eye-glass*, and the one at the greater distance the *field-glass.*

The *positive eye-piece*, of Ramsden is only used in those cases in which it is necessary to see distinctly some object in the eye-piece, as an instrument for measuring, at the same time that the object itself is in focus. In the latter eye-piece the con-

vex surfaces of each of the two glasses are directed towards each other as represented in this diagram.

The *object glasses* used in the best instruments are of English manufacture, but those furnished with the cheap microscopes are made on the continent, and are much less expensive. The defining power of many of these foreign objectives is very good, and they are admirably adapted for all ordinary work—they vary in price from ten to thirty shillings, but a good English quarter of an inch glass cannot be purchased for less than five pounds.

The two most useful object-glasses are the *quarter of an inch* which should magnify from 200 to 220 diameters, and the *inch* which should magnify from 30 to 40 diameters. The definition of these glasses should be good, and they should transmit plenty of light. Any lines in a structure examined by them should appear sharp and distinct. The whole field should be perfectly flat, and every part of it in focus at the same time. The field should not be too small, and there should be no coloured rings round any objects subjected to examination.

Spherical and Chromatic Aberration.—A glass is said to be uncorrected for *spherical aberration* when objects at the circumference of the field are not in focus at the same time as those in its centre, and it is not corrected for *chromatic aberration* if there are coloured fringes around any objects subjected to examination by it. The *defining power* of an object-glass will be found imperfect if it be not properly corrected for spherical and chromatic aberration.

Angle of Aperture.—For ordinary work it will be found inconvenient if the object-glass when in focus comes too close to the object. This is a defect in glasses having a high angle of aperture. The *angle of aperture* is the angle made by two lines from opposite sides of the aperture of the object-glass, with the point of focus of the lens. Glasses with a high angle of aperture admit much light, and define many structures of an exceedingly delicate nature, which look confused when examined by ordinary powers. For general work I recommend you not to use a glass with a higher angle than from 50 to 100 degrees.

Mr. Ross has lately made glasses having an angle of 170 degrees, which are valuable for investigations upon many

Fig. 6.

PLATE II.

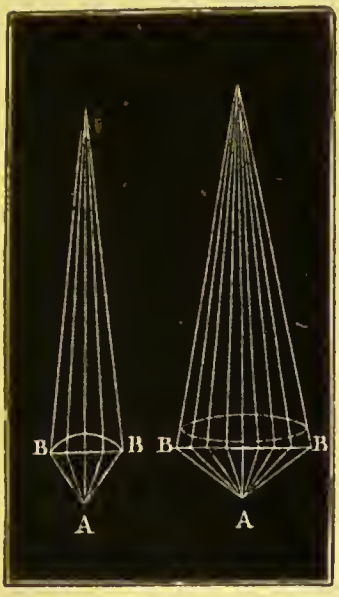


Fig. 7.

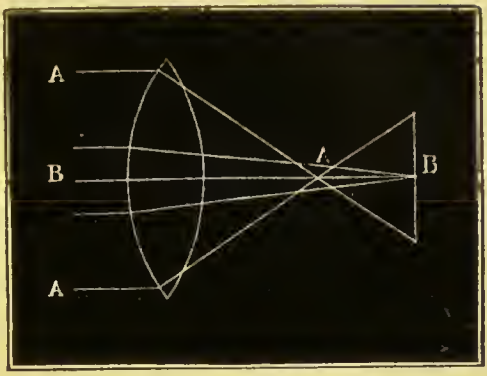


Fig. 8.

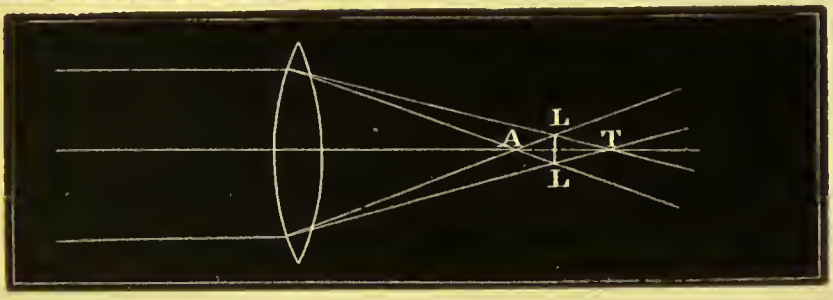


Fig. 9.

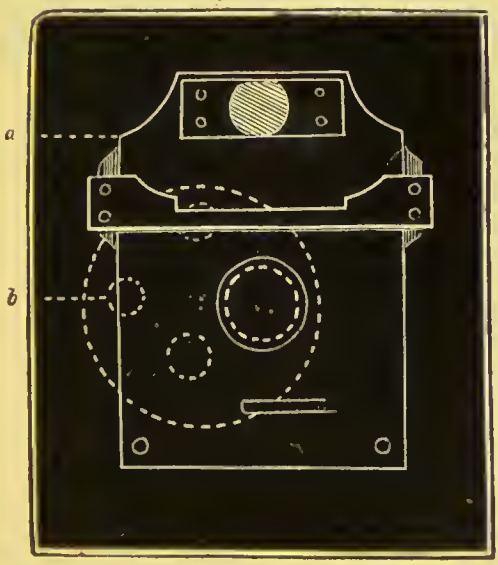


Fig. 10.



Fig. 6. Objective, with low angle of aperture B A B. Another with high angle of aperture B A B (p. 6).
Fig. 7. To illustrate "spherical aberration." The rays A A being more refracted than those near the centre B, are brought to a focus nearer the lens (p. 6.)
Fig. 8. To illustrate "chromatic aberration." The violet and blue rays being most refrangible are brought to a focus, A, nearer the lens than the red rays, T, which are the least refrangible of the rays of the spectrum. Any object placed at L L would exhibit coloured fringes (p. 6).
Fig. 9. Stage of student's microscope, showing diaphragm (p. 17) placed beneath. From *a* to *b* should not be less than two inches (p. 8).
Fig. 10. Mirror.

[To face page 6.]

minute organisms, such as the diatomaceæ ; but these are not required for ordinary work. The importance of arranging the object very carefully, and the necessity of paying great attention to the illumination render these glasses inconvenient for general observation. The *penetrating power* of glasses with a low angle is much greater than in those of a high angle of aperture, so that exact focussing is much more important in the latter than in the former.

The refraction produced by the passage of the light through the thin glass covering the object, varies according to its thickness, and it has been found necessary to render the higher powers capable of being adapted to this variable refraction. It is especially necessary in glasses of high angle of aperture, and is effected by altering the distance between the second and third pair of glasses. This line shows the point to which the lens should be screwed up when adapted for *uncovered objects*, and the other one corresponds to its position for covered objects. In order to adjust the object-glass, it is arranged for an uncovered object, then any object covered with thin glass is brought into focus by moving the body of the microscope ; next, the ring which carries the third lens is screwed round until any particles of dust upon the upper surface of the glass are brought into focus. The glass is then corrected for examining the covered object which may be brought into focus.

The mirror should be capable of movement upon an upright beneath the stage, so that it may be arranged near to, or at a distance from, the object, and it should be capable of being inclined at any angle, so that rays of light may be reflected from it and made to pass directly through the object in straight lines, or thrown upon it in a very oblique direction. The mirror should be of full size, one surface quite plain and the other concave, so that a strong light may be condensed upon the object when required.

Mechanical Portion of the Microscope.

In directing your attention to the mechanical arrangements of the microscope, I must say a few words upon the adjustments for altering the focus, the body of the instrument, and the stage.

Adjustments for Altering the Focus.—The ordinary movement is obtained by the rack and pinion, as in these instruments. In some the body is moved by the fingers alone, and is arranged to slide in a tube like a telescope. In this one, the requisite motion is obtained by a lever, and in this instrument of Mr. Ladd, the focus is adjusted by a chain movement.

Besides these coarse adjustments, however, every microscope should be provided with a more delicate motion for altering the focus when high powers are employed. This *fine adjustment* is differently arranged in detail in various instruments, but is effected by turning a screw having a very fine thread. The movement of Mr. Ladd's chain is so regular and delicate as to supersede the necessity of a fine adjustment.

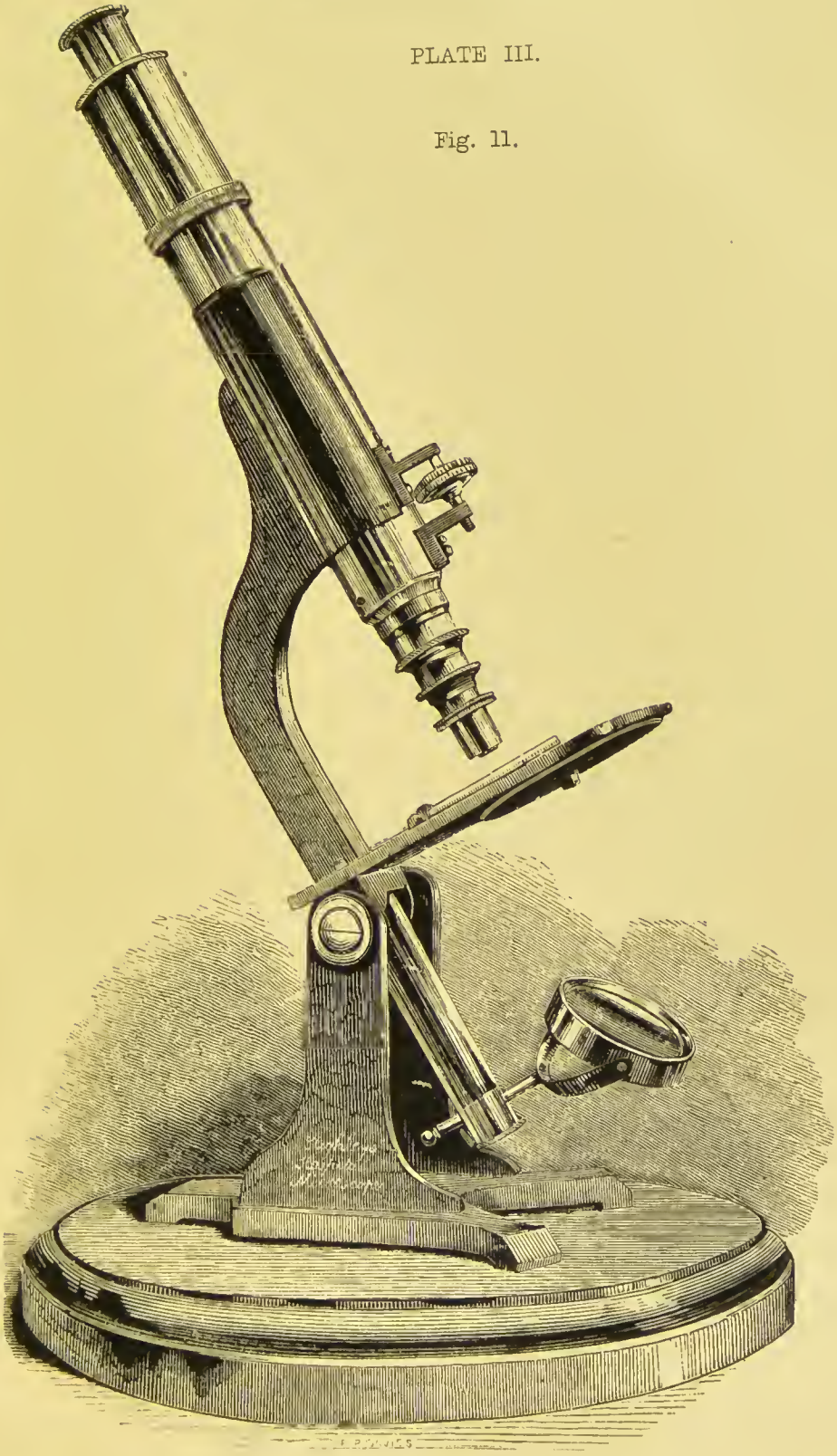
The Body of the Microscope.—The instrument should be perfectly steady, whether the body be inclined or arranged in a vertical position; and not the slightest lateral movement should be communicated to the body of the microscope when the focus is altered by turning either of the adjustment screws. The base or foot should be sufficiently heavy to give steadiness, and should be placed upon three small feet.

The body ought to be provided with a joint by which it may be inclined or placed in a horizontal position, which is required when drawings are made with the camera, or when objects are measured by the aid of this instrument. Another advantage gained by this moveable joint is, that the muscles of the neck do not become so tired when the body of the microscope is inclined, as when the head has to be bent over an instrument standing upright, for several hours at a time. The larger the microscope may be, the more necessary is this joint for the comfort of the observer; and as it in no way impairs the steadiness of the instrument, and only adds a few shillings to the expense, I recommend every one, in the choice of a microscope, to select an instrument which may be placed in a vertical, inclined, or horizontal position. The existence of this joint can do no harm, and if the observer never intends to incline his microscope it is at least desirable that such an alteration in its position should be possible.

The stage should be sufficiently large to admit either edge of a glass-slide, two inches in diameter, to be brought under the object-glass. The stage of the microscopes of Nachet,

PLATE III.

Fig. 11.



Student's microscope, designed by Mr. Highley, and made by Mr. Ladd, on a stand, so that it may be readily covered with a glass shade (p. 10).

[To face page 8.]

Oberhäuser, and others, are too small. The distance from the centre of the object-glass to the upright pillar should not be less than two inches.

Diaphragm.—Beneath the stage a circular diaphragm with holes in it of several different sizes should be so arranged that it can be made to revolve without difficulty and any hole brought under the object; a catch is of great advantage in placing the hole in the centre of the field.

Microscope Makers.—The great number of different microscopes, and the excellent workmanship employed in their construction, render it a difficult as well as a delicate task for a teacher to recommend any special one to his pupils. Indeed, I cannot select any one instrument in preference to others; for although many of the instruments which I have used are exceedingly good, I doubt not that there are others which I have never had an opportunity of testing, which are quite as good in every respect.

The microscope makers whose addresses I have been able to ascertain are here arranged in alphabetical order :—

English Makers.

Baker . . .	Holborn.
Bryson . . .	Princes Street, Edinburgh.
Dancer . . .	43, Cross Street, Manchester.
Field . . .	Birmingham.
King . . .	Bristol.
Ladd . . .	31, Chancery Lane.
Matthews . . .	Portugal Street, Lincoln's Inn.
Pillischer . . .	88, New Bond Street.
Powell and Lealand .	Seymour Place, New Road.
Ross	Featherstone Buildings, Holborn.
Salmon . . .	100, Fenchurch Street.
Smith and Beck .	Coleman Street, City.

Foreign Makers.

Amici . . .	Modena.
Brunner . . .	Paris.
Chevalier . . .	Paris.
Frauenhofer. Merz .	Munich.
Nachet . . .	16, Rue Serpent, Paris.
Oberhäuser . .	Paris.
Pistor. Schick . .	Berlin.
Ploesl . . .	Vienna.

It is, however, due to those makers who have taken the lead in the manufacture of cheap microscopes, that their instruments should be specially referred to. By cheap microscopes I mean instruments which, with two powers, an inch and a quarter-inch, can be sold for about five pounds.

Students' Microscopes.—Mr. Salmon, Mr. Highley, and Mr. Matthews were, so far as I know, the first makers in London who brought out a really good, cheap, practical instrument, furnished with foreign object-glasses. Mr. Highley's pattern is now made by Mr. Ladd.

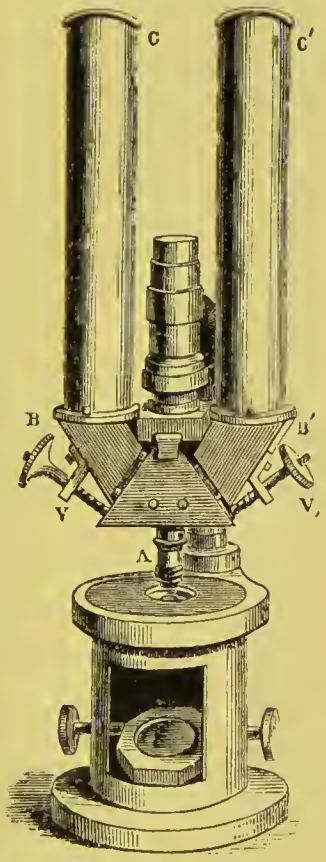
I would strongly recommend all who are about to purchase a student's microscope to examine the instruments of these makers. I can also strongly recommend the educational microscope of Messrs. Smith & Beck, which, however, is somewhat more expensive, costing ten guineas with case.

The microscope made by Mr. Field, of Birmingham, which gained the medal at the Society of Arts, is, for the price, an exceedingly good instrument. It is provided with two eye-pieces, two object-glasses (magnifying from 25 to 200 diameters), bull's-eye condenser forceps and a live box, and packed in mahogany case with this apparatus complete, costs only three guineas.

Those who wish for a microscope as perfect as it can be made in the present day I should advise to look at the beautiful instruments of Powell and Lealand, Ross, and Smith and Beck. I would also recommend those of Mr. Pillischer to attention. In alluding specially to these instruments I wish it to be distinctly understood that I do not in any way disparage the work of other and less celebrated makers, but having had some experience with those instruments to which I have directed your attention I feel it right to express an opinion upon them.

In choosing a microscope the following requirements should be borne in mind:—With reference to the optical part, the *inch object-glass* should magnify not less than 30 diameters, and the *quarter* not less than 200, when the *shallow eye-piece* is applied. The *field* should be well lighted, and the lines of delicate objects submitted to examination should be sharp and well defined. The mirror should be large (at least two inches in diameter), one side plane, the other concave, and it should

Fig. 12. PLATE IV. Fig. 13.



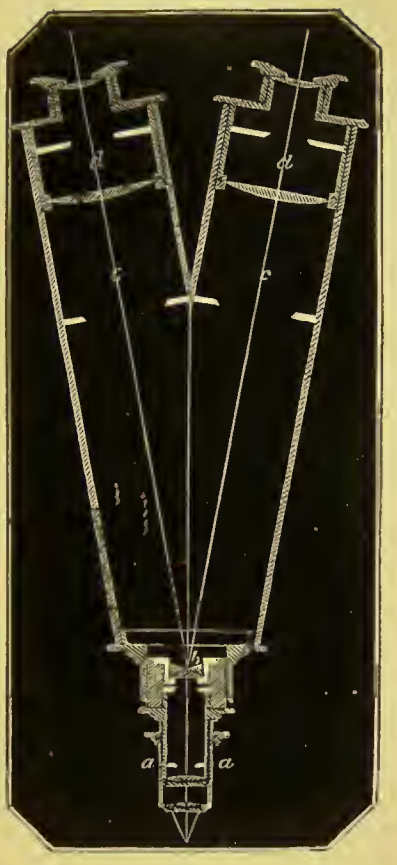


Fig. 14.

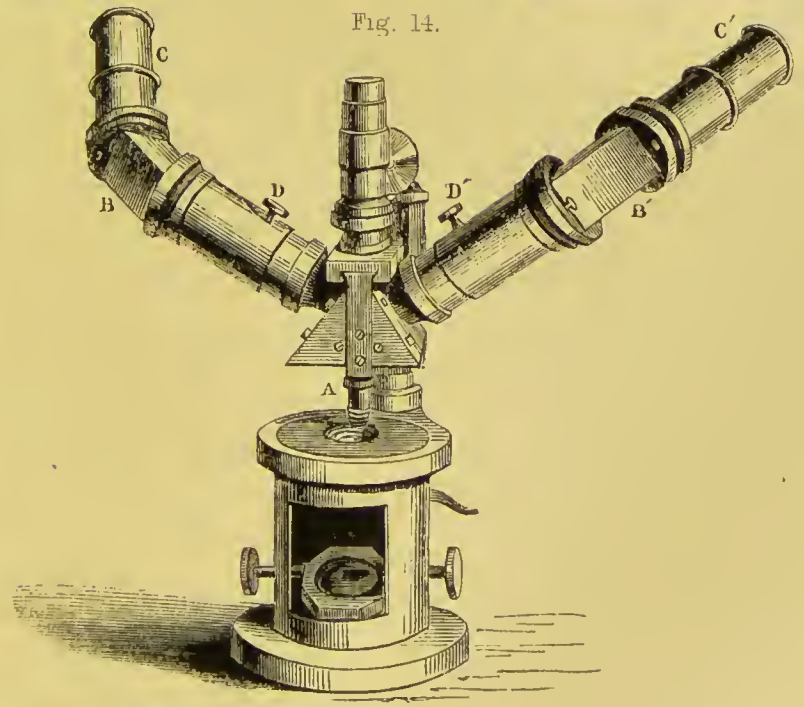


Fig. 12. Nachet's binocular microscope (p. 11).
Fig. 13. Binocular microscope as arranged by Mr. Wenham.
Fig. 14. M. Nachet's microscope to enable two observers to examine an object at the same time.

[To face page 10.]

be adapted to the body of the microscope in such a manner that very oblique rays of light may be made to impinge upon the object.

With regard to the mechanical portion of the microscope, the adjustments should work smoothly, and an object placed in the field for examination should not appear to move or vibrate when the screws are turned. The body should be provided with a joint, so that it may be inclined or placed quite horizontally. The stage should be at least three inches in length, by two and a half in width, and there should be a distance of at least an inch and a half from the centre of the opening in the stage over which the slide is placed, to the upright body. The motion of the slide upon the stage, and all other movements and adjustments, should be smooth and even, without any tendency to a jerking or irregular action.

Necessary Apparatus.—Every student's microscope should be provided with a neutral tint glass reflector for drawing and measuring objects, a diaphragm, to the under part of which is fitted a tube to receive an achromatic condenser, or polarizing apparatus, a bull's-eye condenser, one shallow eye-piece, and two powers—a low one, magnifying from 20 to 40 diameters, and a quarter of an inch which magnifies at least 180 diameters.

These instruments should be conveniently packed in the case with the microscope. The polarizing apparatus and the achromatic condenser can be purchased afterwards. The cost of the microscope without these instruments, but including the other apparatus mentioned, should not be more than from six to seven pounds; and if the microscope be mounted upon a cast-iron foot instead of a brass one, it may be obtained for about a pound less, without its practical utility being in any way impaired.

Binocular Microscope.—M. Nachet has succeeded in making a binocular microscope in which objects appear in relief, but unfortunately the effect is not satisfactory with high powers. Doubtless, ere long improvements will be effected, and we may hope to be provided with an instrument having this most desirable power.

Travelling and Dissecting Microscopes.—For travelling, and especially for sea-side work, it will be convenient to be pro-

vided with a microscope which can be packed in a smaller compass than the instruments before alluded to.

Mr. Warington, some time since, designed a very simple microscope for travelling purposes. The stand consists of two flat pieces of oak, fitted at right angles to each other by means of pegs. The stage is inserted into the longer one, to the top of which the body of the microscope is adapted by means of a clamp, in which a horizontal bar carrying the body can be moved backwards and forwards. This instrument can be arranged in an upright or standing position, and by means of the clamp the body can be attached to a table, so that living objects in upright glasses can be subjected to examination. In its present form, however, the instrument is not so steady as could be wished, but by a slight modification in its structure it could probably be made more so.

Another simple form of travelling microscope is described by me in the fourth volume of the Transactions of the Microscopical Society (page 13).

This instrument is made entirely of tubes, is very steady, and can be used in any position. It makes an excellent microscope for dissecting, and the alteration of focus is effected very rapidly by means of a knee lever, which was kindly made for me by Mr. Becker, instead of a screw.

This microscope takes to pieces, and can be packed in a very small case. Its structure is so simple that it cannot easily get out of order. The legs of the tripod stand have been made with hinged joints by Mr. Matthews, which diminishes the bulk of the instrument when packed up to a still greater degree. The price, however, of this microscope is £5 (although I believe it could be well made for considerably less), while that of Mr. Warington can be purchased for half that sum.

Large Microscopes.—The large expensive microscopes are provided with every instrument which modern science has placed at the disposal of the observer. For delicate investigations many of these are invaluable, but for ordinary work they are not necessary, and their expense is so great as to place them beyond the reach of the great majority of observers. You may examine presently the beautiful microscopes by Powell and Lealand, Pillischer, Ross, and Smith and Beck, which have been placed on the table. Very expensive and

Fig. 15.

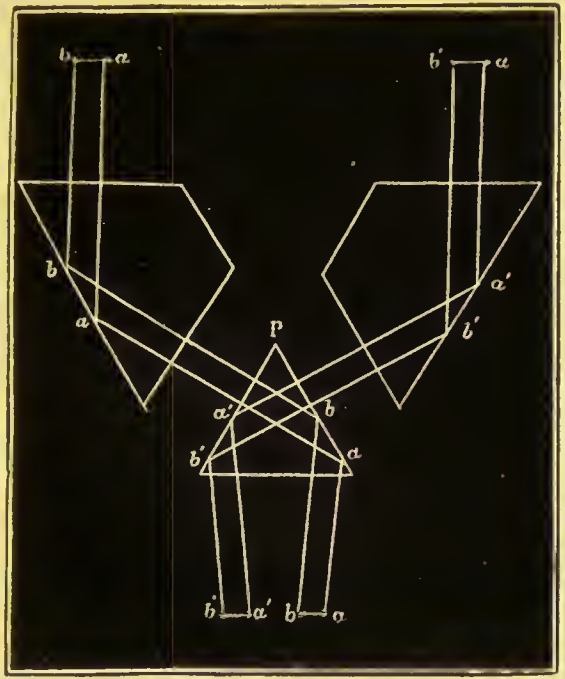


Fig. 16.

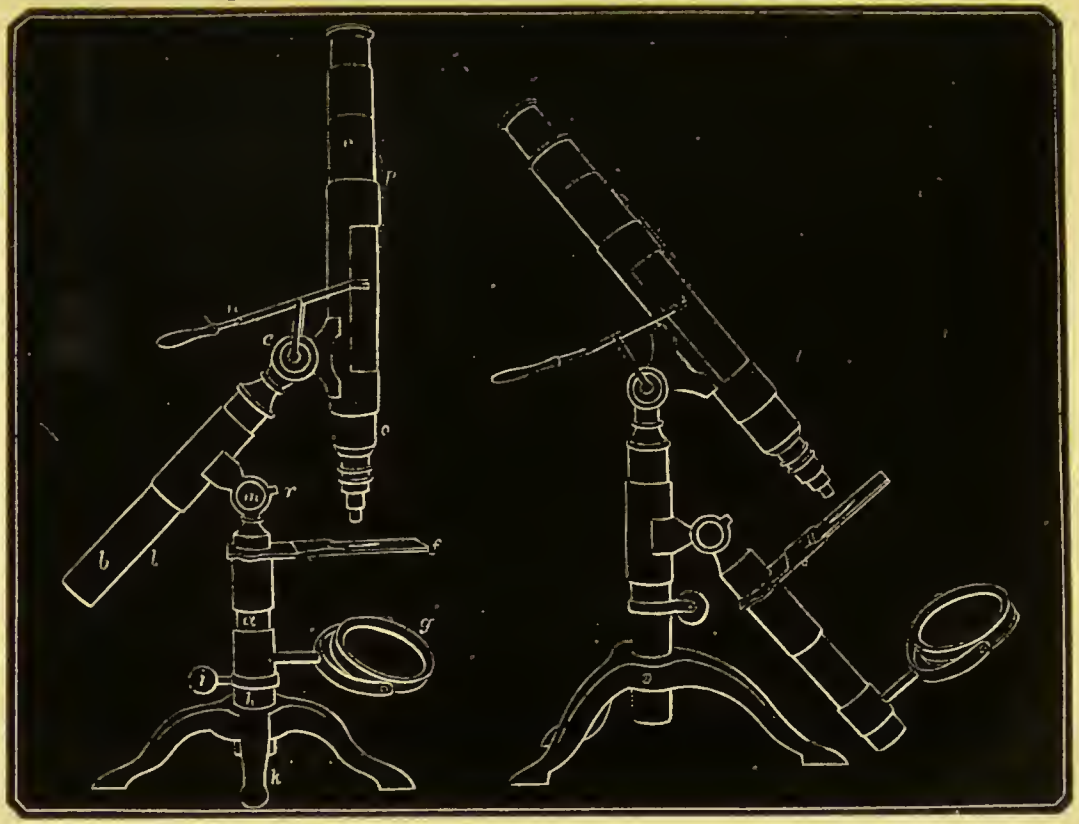


Fig. 17.

Fig. 15. Prisms in Nachet's binocular microscope. Figs. 16 and 17. Travelling microscope. *a*. Telescopic stem of the microscope made of brass tubes an inch in diameter. *b*. Horizontal arm of ditto. *c*. Body. *e*. Hinge joint. *f*. Stage. *g*. Mirror. *h*. Tube with clamp screw, *i*, in which the lower part of the stem slides. *k*. Lower part of stem, to which the mirror can be adapted. *l*. Ridge which prevents the horizontal bar from turning round. *m*. Hinge joint. *n*. Knee lever for adjustment. *o*. Fine adjustment screw. *p*. Pin which prevents the body being forced lower than the focus of the object-glass.

[To face p. 12.]

PLATE VI.

Fig. 19.

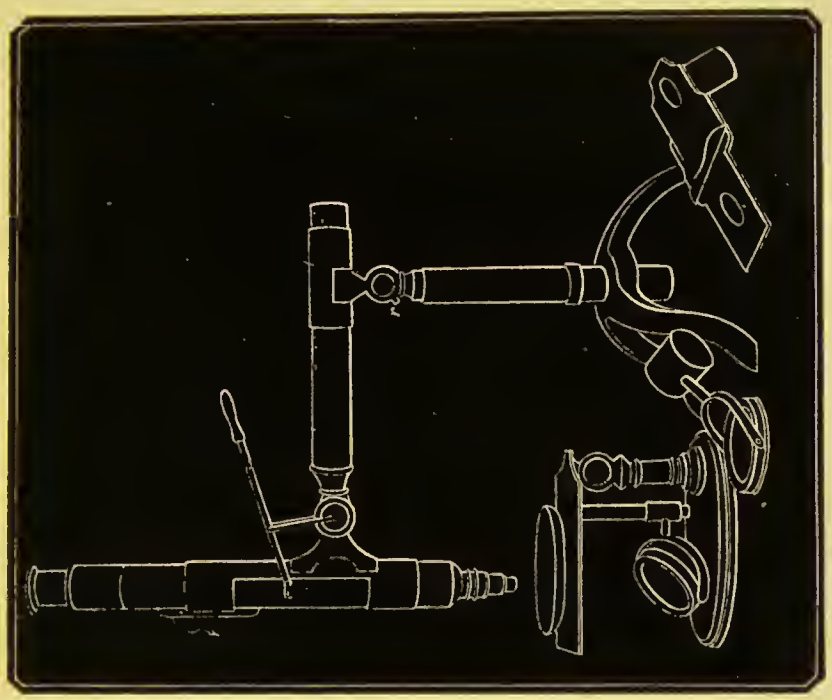


Fig. 19. Same instrument arranged for making dissections.

Fig. 18.

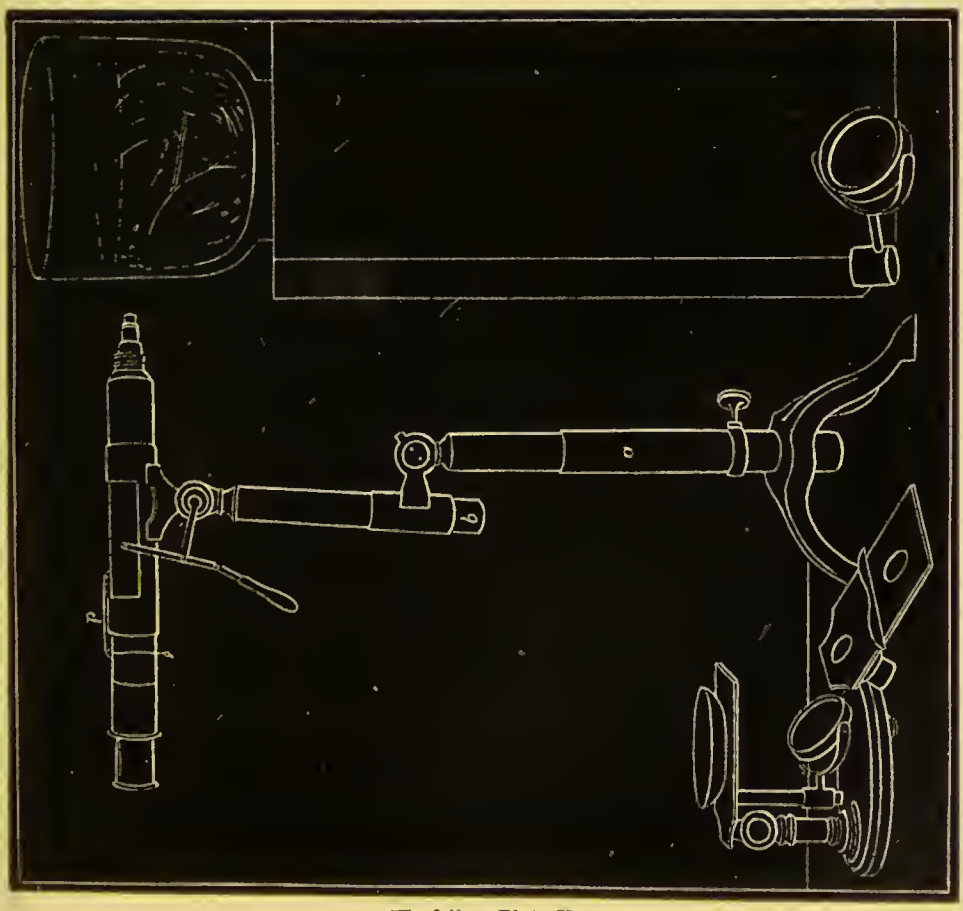


Fig. 18. The travelling microscope arranged for the examination of objects in a vivarium.

[To follow Plate V.]

delicate instruments are required so seldom in ordinary work, that most observers will be able to examine any special preparations under the instrument of a friend, whenever such very minute examination is necessary.

LECTURE II.

Examination of objects by reflected light, transmitted light, and polarized light.—*Reflected light.*—Different methods employed. —By bull's eye condenser or side reflector.—By Lieberkuhns —Dark-ground illumination.—Parabolic reflector.— Annular condenser. — *Transmitted light.* — Diaphragm.— Achromatic condenser—Gillett's condenser.—*Polarized light.*—Polarizer.— Analyzer.—Iceland spar.—Tourmaline.—Iodo-quinine or Herapathite.—*Illumination by artificial light.*—Lamps.—Camphine lamp.—Oil lamps.—Argand lamp.—French moderator lamps.— Gas lamps.—Of the importance of protecting the eyes from the diffused light of lamps.—*On drawing microscopical specimens.* —Camera lucida.—Steel disc.—Neutral tint glass reflector.— *On measuring objects.*—Cobweb micrometer.—Stage micrometer. —Directions for measuring objects.—Measuring the angles of crystals.—On ascertaining the magnifying power of object-glasses.

Examination of Objects by Reflected Light, Transmitted Light, and Polarized Light.

Gentlemen,—From the most cursory examination of objects, we discover that their internal structure differs materially in character from the external surface, and if we examine the internal arrangement as well as the external surface of an object, we shall be led to form an idea of its nature very different to that which we should have arrived at, if we regarded one set of characters only. Again, by employing polarized light we may often make out points in the structure

of an object which cannot be perceived when it is examined by ordinary light.

I must therefore direct your attention to the three following methods of directing the light upon objects submitted to microscopical examination.

1. *Reflected Light*, in which the light is thrown down upon the object, and the peculiarities of its surface alone observed, as in looking at different objects under ordinary circumstances.

2. *Transmitted Light.* The second mode of examination is by the aid of transmitted light by which any inequalities in the internal structure of an object are demonstrated.

3. *Polarized Light.* By means of which the internal structure of various transparent objects may be rendered evident, although they may not be recognizable by ordinary illumination.

Reflected light may be employed for the examination both of transparent and opaque objects, but transmitted light is only adapted for the examination of transparent structures. Every object to be examined by transmitted light should be very thin, or must be rendered transparent by some special method of preparation. To view an object by reflected light, the light must be thrown down upon it from above, by employing either the direct rays from a luminous body, or by the aid of a reflector as shown in this microscope ; but in order to see the internal structure of a transparent object by transmitted light, the light must be so placed that the rays can pass directly through it, or they must be reflected upon its lower surface from a mirror placed beneath it, and arranged at the proper angle.

Reflected Light.

The light employed may be ordinary *daylight*, *sunlight*, or the *light* of a *candle*, or *good lamp*. The most important modes of illuminating objects for examination by reflected light are the following :—

1. By ordinary *diffused* daylight, sunlight, or lamplight, but diffused light will usually be found insufficient for good illumination.

2. By condensing the light upon the object by means of a large simple plano-convex lens, or *bull's-eye condenser*, or when

PLATE VII.

Fig. 20.

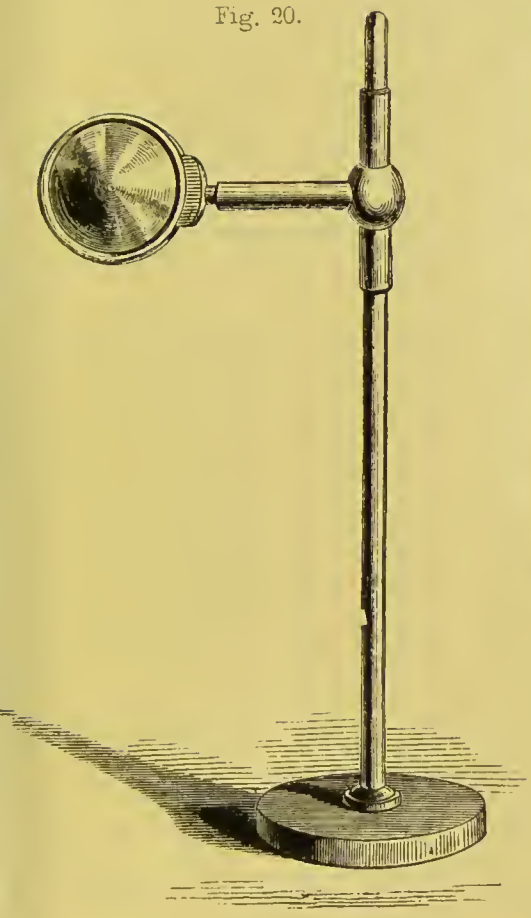


Fig. 22.

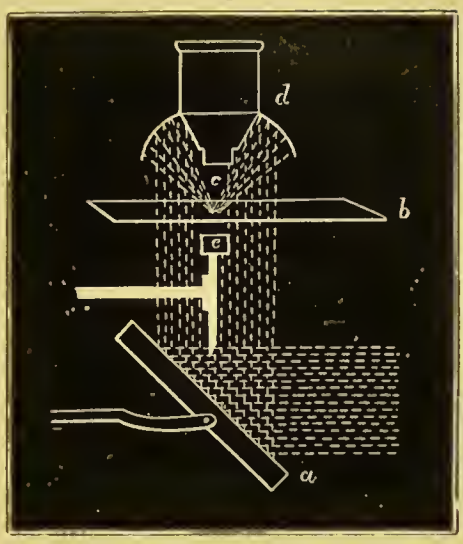


Fig. 23.



Fig. 21.

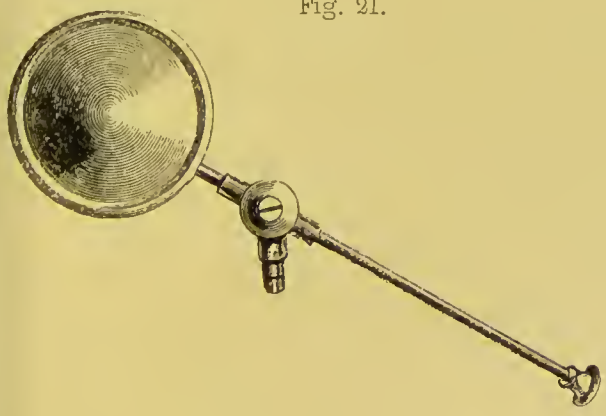


Fig. 20. Bull's-eye condenser for condensing a strong light upon objects. Fig. 21. Bull's-eye condenser to fit on to the stage (p. 14).

Fig. 22. To illustrate the mode of examining an object by reflected light with the Lieberkuhn. The light reflected from the mirror, *a*, passes through the glass slide, *b*, around the object, and impinges on the concave annular mirror, *d*, by which the rays are brought to a focus and condensed upon the object placed at *c* (p. 15).

Fig. 23. Forceps to fix upon the stage for holding small objects.

[To face page 14.]

very high powers are required, by a combination of two lenses, conveniently arranged.

3. By causing the light to be brought to a focus upon the object by means of a small concave *metallic reflector* fitted upon one side of the instrument.

4. By causing the rays of light reflected from the mirror and passing round the *circumference of the object* to impinge upon a *concave annular reflector* or *Lieberkuhn* adapted to the object-glass, from which the rays are reflected downwards, and brought to a focus upon the surface of the object itself. The light is prevented from passing directly through the object by a small metal stop, or even by a piece of black paper placed immediately beneath it, and corresponding to the aperture of the object-glass. This arrangement will be readily understood by referring to this diagram, or by noticing the microscope which has been arranged for examining an object in this manner.

The *first* mode of illumination seldom affords sufficient light to show the character of the surface satisfactorily. The *second* and *third* plans are those usually adopted, and are the most convenient as well as the most efficient modes of illuminating the surface of objects. The *fourth* method is now seldom resorted to, and is only applicable in cases where the object is small enough to permit the passage of a sufficient quantity of light around it. If mounted as a transparent object, a piece of black paper, rather larger than the aperture of the object-glass, should be placed behind it to prevent the passage of light through it, or one of the stops supplied with some instruments may be inserted in its place beneath the stage. The stops, however, are not furnished with most of the modern microscopes as the other modes of illumination, and those next to be described, afford the most satisfactory results.

Dark-Ground Illumination. In this place I must allude cursorily to a mode of illumination which has been much in repute of late years, and which is very advantageous for demonstrating some structures. I refer to *dark-ground illumination*, in which the object appears in relief upon a black ground. This mode of illumination is particularly applicable to investigations upon the most minute organisms, such as the diatomaceæ. The appearance produced is very different

to that obtained by merely throwing the light upon the surface of the object, and many points may be learned with reference to the nature of the markings upon a specimen which could not be ascertained by the ordinary methods of directing the light upon it. In this mode of illumination the direct rays are prevented from penetrating the specimen, and passing through the object-glass, but the preparation is highly illuminated upon all sides by light made to impinge upon it in a very oblique direction. Thus the object is thoroughly illuminated at all points, but the ground on which it lies appears perfectly dark. There are several methods by which this result may be obtained. This little instrument which I now show you is termed a *spot-glass*,* and consists of a plano-convex lens, the convexity being so great that rays of light passing through it would be made to converge with a great degree of obliquity, and would be brought to a focus at a short distance above the flat surface of the lens. In the centre of the flat surface is placed a small circular piece of black paper in order to prevent the passage of any direct rays of light. The lens is fixed in a brass tube made to slide up and down, so that it may be adjusted at the proper distance below the object.

The *parabolic reflector* of Mr. Wenham, Mr. Shadbolt's *annular condenser*, and the *parabolic illuminator* of Messrs. Smith and Beck, are beautiful instruments for effecting the same purpose in a more efficient manner. Another excellent plan has lately been devised by Mr. Wenham, the simplicity of which recommends it strongly to our attention. A small triangular prism is placed beneath the object, so that one of its plane surfaces is in contact with the under surface of the slide carrying the object. The light is refracted so highly that none passes directly through the object; but being thrown at the proper angle upon the under surface of the thin glass which covers the object, is entirely reflected from thence upon the object itself, which is thus highly illuminated.

In employing these instruments the light should be reflected from the plane mirror.

I must not detain you longer upon this matter of dark-

* The spot-glass may be obtained of the different microscope makers for about 7*s.* 6d.

PLATE VIII.

Fig. 25.

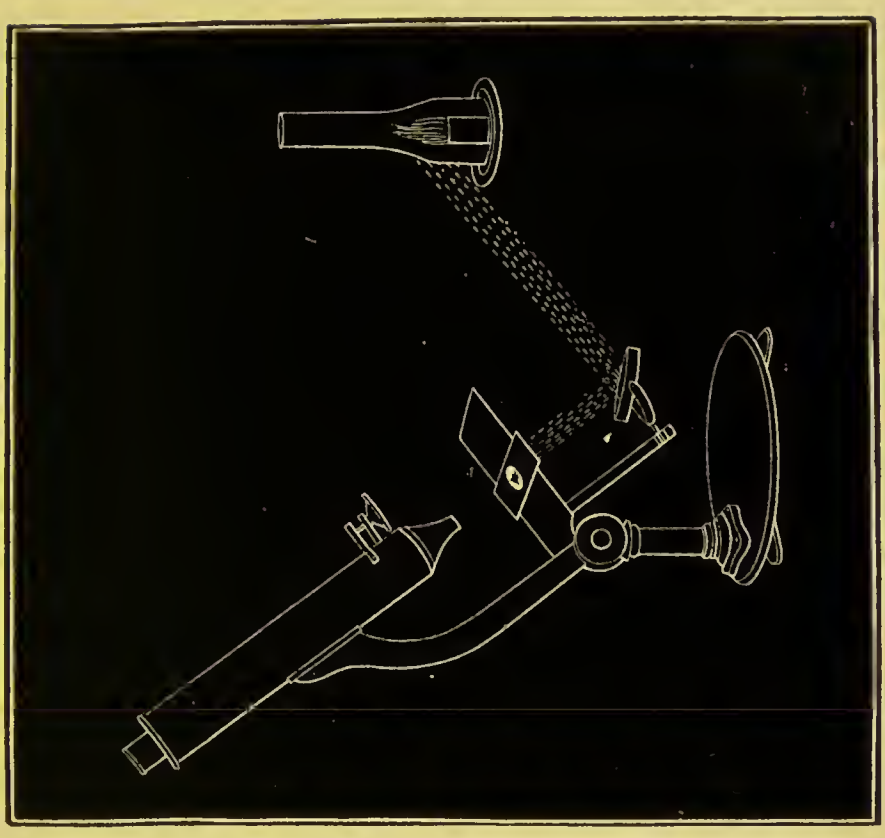


Fig. 25. Diagram to show the arrangement for examining objects by transmitted light (p. 17.)

Fig. 24.

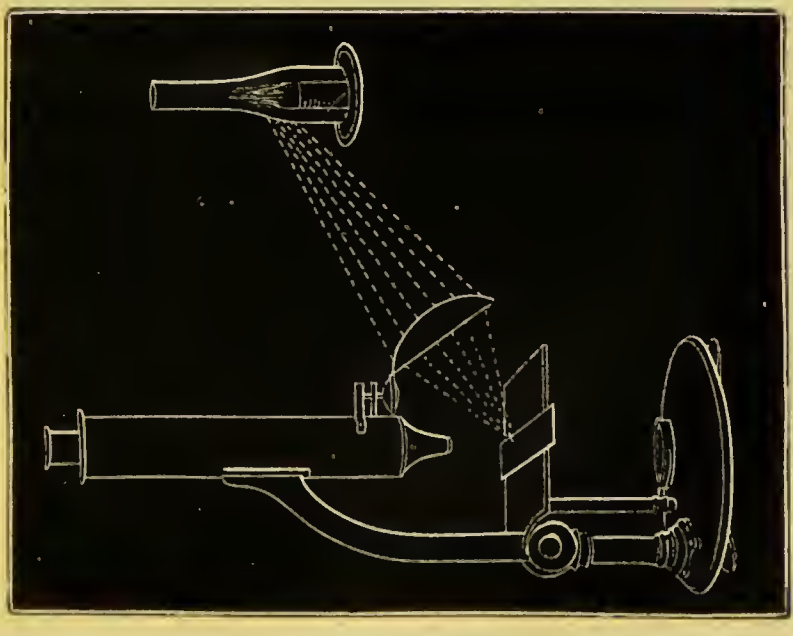


Fig. 24. Diagram to illustrate the arrangement for examination by reflected light (p. 14).

[To face page 16.]

ground illumination, but must refer those who are interested in the subject to some of the works previously alluded to, especially to the treatises of Professor Quekett and Dr. Carpenter.

Transmitted Light.

In discussing the mode of illuminating objects by transmitted light, I shall have to draw your attention to two or three beautiful instruments for condensing the light upon the object. This microscope is arranged in the ordinary position for examining transparent objects. The light may be received upon the plane or concave mirror, according as a moderate or brilliant light is required, but as a general rule, the intensity of light should not be greater than necessary to make out distinctly the structure of the object. Direct sunlight is not to be employed, and a very strong light of any kind is hurtful to the eyes. The best light during the day is to be obtained from a white cloud upon which the sun is shining.

Diaphragm.—Beneath the stage in these microscopes you may observe a plate with holes in it of different sizes. This is the diaphragm, and is employed for cutting off the most oblique rays and superfluous light. Every microscope should be provided with a diaphragm fitted on about an inch beneath the stage, and arranged on a pivot, so that any of the holes may be brought under the object. The definition of the structure of a transparent object is often found to be very much clearer when only the more direct and central rays of light from the concave mirror are allowed to pass through it.

Achromatic Condenser.—The illumination of objects examined with high powers is much improved by causing the light to pass through an achromatic condenser which consists of an ordinary achromatic objective of half or a quarter of an inch focus, arranged in a sliding tube immediately beneath the stage. One of these instruments can be fitted to the student's microscope. Mr. Quekett has adapted a simple lever handle by means of which the right focus is readily obtained. Every microscope which is employed for delicate observation should be provided with an achromatic condenser. The instrument is not an expensive one if it be made of a French combination.

Gillett's Condenser.—Mr. Gillett has adapted a diaphragm plate and stops to the achromatic condenser, and there is a beautiful instrument of this kind made by Mr. Ross. Messrs. Powell and Lealand have, however, improved upon it, and brought out a much smaller and more compact condenser, which is attached to their microscope.

Polarized Light.

In examining an object by polarized light, it is necessary to have one crystal beneath the object, termed the *polarizer*, and fitted under the stage, and another one above the stage, inserted into the tube above the object-glass, or adapted to the eye-piece—this is termed the *analyzer*.

Various crystalline substances are employed for polarizing the light. Two crystals of *Iceland spar* are usually preferred. These are divided obliquely, and connected together again with Canada balsam so that one of the two images formed by this double refracting crystal is removed from the field of view. *Tourmaline* crystals have also been used, but their colour is a disadvantage.

By the kindness of Dr. Herapath I am enabled to show you two crystals of the *Iodo-quinine*, or *Herapathite*, prepared by him for polarizing the light. These are mounted between two pieces of thin glass, and you will see how well they effect the object for which they are intended, by turning one of them placed in front of the other before a light.

In these four microscopes are placed specimens of the same object, subjected to examination in a different manner in each instance, and you will not fail to be surprised at the different appearance of the object in each case. In this one the surface of the object is examined by *reflected light* brought to a focus upon it by means of a bull's-eye condenser. In the second instrument, the light is *reflected* upon it from a *Lieberkuhn*. In the third, the light is transmitted through the object after having been reflected from the surface of the mirror ; and in the fourth, the object is seen under the influence of *polarized light*. Now I must ask you to notice carefully the appearances you observe in each case, and after comparing them with each other, consider the probable nature of the

PLATE IX.

Fig 26.

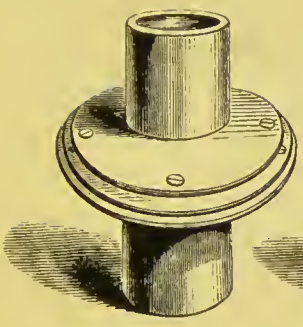


Fig. 27.

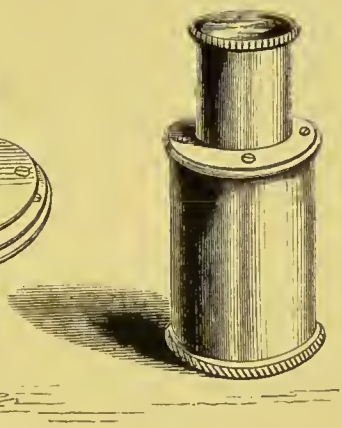


Fig. 28.



Fig. 29.

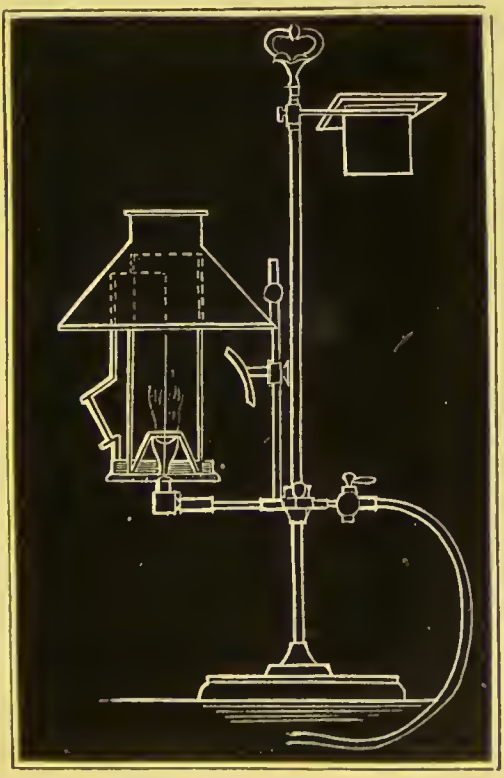


Fig. 30.

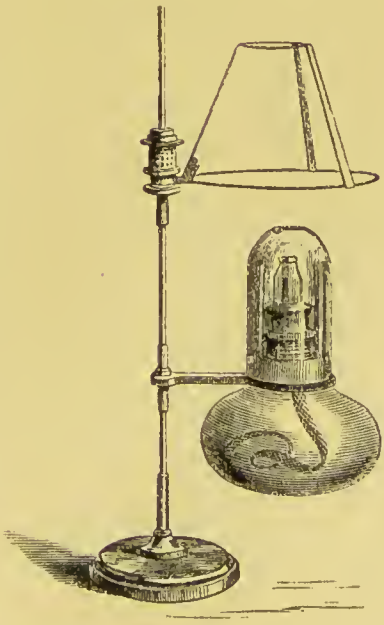


Fig. 26. "Polarizer" placed beneath the object. Fig. 27. "Analyzer" placed above the object.
Fig. 28. Nicol's prism divided and cemented together with Canada balsam, in order that one image produced by the double refracting spar may be refracted out of the field of vision (p. 18).
Fig. 29. Gas lamp arranged by Mr. Highley with water bath and hot plate (p. 19).
Fig. 30. Camphine lamp arranged by Messrs. Smith and Beck (p. 19).

[To face page 18.]

substance, and the precise arrangement of the elementary parts of which it is made up. Lastly, compare the conclusion to which you have arrived with reference to the nature of the structure after submitting it to these several modes of examination, with the idea you would have been led to form of it from an observation made by either mode of illumination separately.

Illumination by Artificial Light.

It may be said with truth, that microscopical work should if possible be undertaken only by daylight, since the most perfect artificial light which can be obtained is far inferior for delicate observation, while it strains the eyes very much more. Still many of us are compelled by necessity to work principally by night, and it is therefore a matter of the greatest importance to be provided with the best kind of artificial illumination.

Lamps.—From time to time various microscope lamps have been proposed. The small *camphine lamp* of Messrs. Smith and Beck represented in this diagram, is the most perfect which I have seen. It gives a beautiful white light, and produces very little heat. Of *oil lamps* there are several which serve for microscopical examination. The *German Argand lamp* lately imported into this country by Mr. Pillischer, is an excellent miscroscope lamp, and so also is the *French moderator*, especially if provided with a blue or neutral tint glass, and a shade.

Gas Lamps.—For those provided with gas I recommend very strongly the gas lamp of Mr. Highley, which is provided with a flat brass plate and a water bath, instruments of great use in microscopical investigation. The light is made to pass through an opening in a diaphragm, and the eyes are quite protected from the diffused light. A very pleasant light is produced by causing the rays to be transmitted through a blue chimney glass and a flat piece of neutral tint glass. The only objection to this lamp is its great heating power. One of these lamps is placed on the table.

In all cases the eye should be carefully protected from the dazzling light when not employed in looking through the

instrument. The eye not observing, should always be kept open, but should be protected from the direct glare of the microscope lamp. For this purpose a shade made of black paper may be fitted to the body of the instrument at a convenient distance below the eye piece. In all cases the light should be perfectly steady, and so situated that it may be conveniently reflected upon the object by the mirror.

On Drawing Microscopical Specimens.

In delineating an object magnified by the microscope, it is important to copy it correctly, both as regards the relative position of the several structures to each other, and also with respect to size. To copy the size exactly will be found extremely difficult by the eye alone, but there are several ways of proceeding by which accuracy may be ensured. Some of these I shall now briefly describe. The simplest method is to place the paper upon the same level as the stage upon which the object is situated. If we now look steadily at the object with one eye, while the other governs the movements of the pencil upon the paper, the object will appear to be thrown as it were upon the paper, and its outline may be very readily traced. By a little practice the relative size of objects may be insured in this manner, but it is troublesome and difficult to keep both the object and paper perfectly still.

Camera lucida.—The principle of the camera lucida has been applied to taking microscopical drawings, and has been found to succeed admirably. The object appears to be thrown down upon the paper, and with a little practice the observer may trace the lines with great accuracy.

Steel Disc.—If a little steel disc be placed at an angle of 45° with the eye-glass, it will receive the magnified image of the object and reflect it upwards upon the retina of the observer. The disc is smaller than the aperture of the pupil, and the pencil can at the same time be seen very well as it traces the image apparently thrown down upon the paper beneath.

Neutral Tint Glass Reflector.—The simplest and cheapest reflector for microscopical drawing consists of a small piece of plate-glass slightly coloured, in order to improve its reflecting power, but still not so dark as to prevent an object being seen

PLATE X.

Fig. 31.

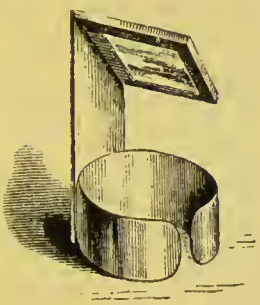


Fig. 32.

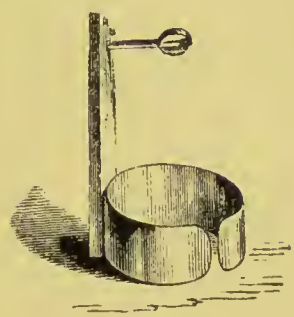


Fig. 33.

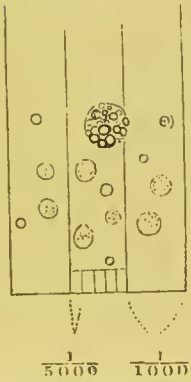


Fig. 34.

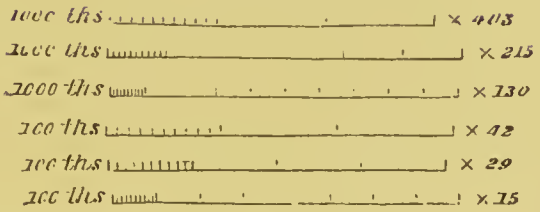


Fig. 35.

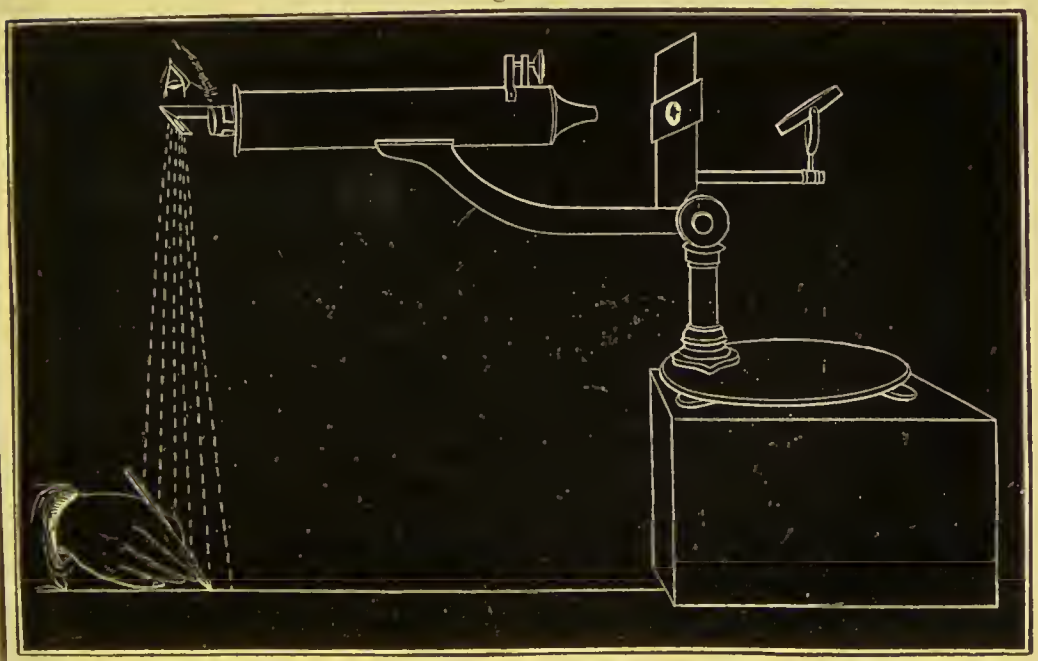


Fig. 31. Neutral tint-glass reflector arranged by Messrs. Powell and Lealand. Fig. 32. Steel dise (p. 20).
Fig. 33. Stage micrometer divided into 1000ths and 5000ths of an inch, magnified 215 diameters.
Fig. 34. 100ths and 1000ths of an English inch magnified in various degrees. The smallest divisions are 1000ths and 10000ths (p. 22).
Fig. 35. To illustrate the arrangement of the microscope for drawing and measuring objects (pp. 21—23).

[To face page 20.]

through it perfectly. This is also arranged at an angle of 45° with the eye-glass, and the draughtsman can very easily follow his pencil upon the paper.

It is important, however, in using these instruments, to arrange the light carefully. The image should not be illuminated too intensely, and the paper upon which the drawing is made should not be too dark, or the point of the pencil will not be seen very distinctly. Experiment can alone decide the relative intensity of the light upon the object and upon the paper, but with a little practice the proper amount of illumination will be discovered. The distance between the reflector and the paper should be precisely the same as from the object to the eye-piece, for otherwise the size of the object delineated will be altered.

This microscope is arranged for copying an object in the manner just described. The object appears to be thrown upon the paper, and now I can trace its outline very readily. If I require to draw it smaller, I need only place the paper upon a stand closer to the reflector. If, on the other hand, I wish to magnify the object so as to draw a *diagram*, I have only to increase the distance. By placing the diagram paper upon the floor, thus, with a long pencil I can readily trace the object. In this manner these three diagrams have been made. They must be of course accurate copies of the objects themselves, and are therefore far more truthful than diagrams representing microscopical structure usually are.

On Measuring Objects.

Most of the larger and complete microscopes are furnished with micrometers adapted to the instrument, but it appears to me that the simple method of measuring objects, presently to be described, to a great extent supersedes the necessity of those more expensive arrangements. It will be well for me, perhaps, in the first place, to describe briefly the different forms of micrometers in use.

The Cobweb Micrometer, originally applied to telescopes by Ramsden, its inventor, is a beautiful instrument which can be fitted to the upper part of the body of the microscope. A fixed cobweb crosses the field of view, and parallel to this is

another cobweb thread capable of being brought near to, or separated from the first, by means of a milled head, to which is attached a graduated circle. The value of each degree on the circle is ascertained by placing an object of known dimensions, as the *stage micrometer* graduated to thousandths, under the object-glass, and ascertaining the number of degrees on the screw which correspond to the 1–1000th of an inch. From these data a simple table may be constructed, and the diameter of any object can be readily ascertained by bringing one side of it up to the fixed line, and causing the moveable line to touch the opposite. If we ascertain the value of the degrees as marked upon the circle when the lines are separated at the proper distance, we may estimate directly the diameter of the object. The older observers used to measure objects by means of very delicate wires, separated from each other by certain known distances placed in the focus of the eye-piece, or by employing points, one of which could be moved from or towards the other by means of a screw.

Stage Micrometer.—Within the last few years, lines, separated from each other by certain known but very minute intervals, have been ruled upon slips of glass by means of a diamond attached to a beautiful instrument, provided with a most delicate arrangement for moving it the required distance from the last line engraved. A second line is then ruled, then a third, and so on. Excellent stage micrometers of this kind have been ruled by Mr. Jackson.

To such wonderful perfection has this process been carried that M. Nobert, of Griefswald, in Prussia, has engraved lines upon glass so close together that upwards of 50,000 would go in the space of an English inch. Several series of these lines were engraved upon one slip of glass, by using which the defining power of any object-glass could be correctly ascertained. As test objects they are equal to, and even rival, many natural objects which have hitherto been employed for this purpose.

The delicate lines on some of the diatomaceæ are separated from each other by the 1–50,000th of an inch, while the finest lines engraved by M. Nobert are not more than the 1–100,000th of an inch apart.

In order to measure the diameter of an object the glass slide

PLATE XI.

Fig. 36.

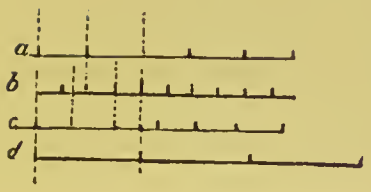


Fig. 37.



Fig. 38.

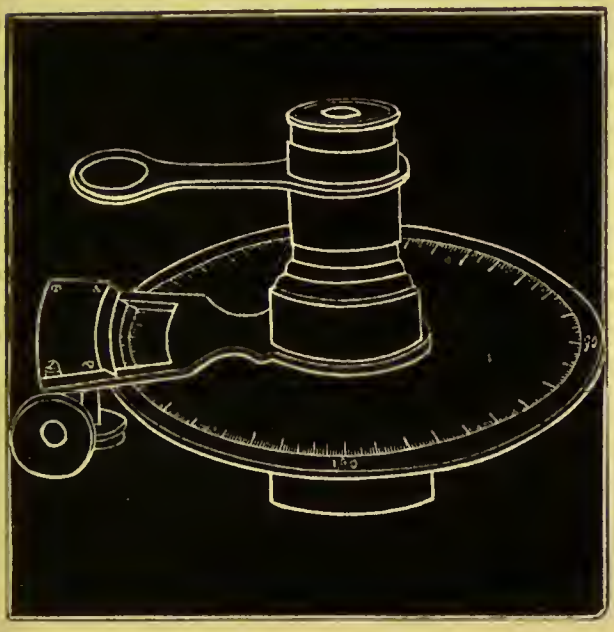


Fig. 39.



Fig. 40.

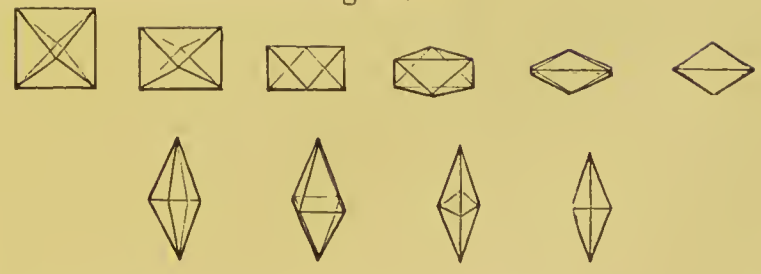


Fig. 36. Mode of ascertaining the magnifying power of an object-glass. *a.* 1000ths of an inch × 200. *b. Inch scale divided into tenths.* *c.* 1000ths of an inch × 130. *d.* 100ths of an inch × 40. Each magnified 1000th of an inch covers two-tenths, or one-fifth of an inch, therefore the glass magnifies 200 times, for $\frac{1}{1000} \times 200 = \frac{2}{10}$ or $\frac{1}{5}$ of an inch. Each 100th of an inch covers four-tenths of an inch, therefore the glass magnifies 40 times, for $\frac{1}{100}$th $\times$ 40 $= \frac{4}{10}$ (p. 24).

Fig. 37. Crystals exhibiting acute and obtuse angles.

Fig. 38. Goniometer for measuring the angles of microscopic crystals (p. 23).

Fig. 39. Achromatic condenser with arrangement for altering focus, designed by Professor Quekett (p. 17).

Fig. 40. An octohedral crystal, one axis of which is shorter than the other two, to show the different appearance it exhibits when examined in different positions.

[To face page 22.]

upon which the lines have been engraved (1-1000th or 1-100th of an inch apart according to the magnifying power) may be placed beneath the object upon the stage. This arrangement, however, is only suitable for low powers, since the object and lines cannot be in focus at the same moment, so that it is impossible to obtain a very correct measurement.

Mr. Jackson arranged a micrometer slide in the eye-piece so that it could be brought over the magnified image of the object by means of a screw.

Simple Method of Measuring Objects.—The most simple and efficacious manner of measuring objects is with the aid of the camera or neutral tint glass reflector referred to before. In the field of this microscope is placed an ordinary micrometer, with the lines separated by thousandths of an inch. Care being taken that the instrument is arranged at the proper distance from the paper, these lines magnified by a quarter of an inch object glass are carefully traced. I remove the micrometer and replace it by the object whose diameter I desire to ascertain. The object is traced over the lines, or upon another piece of paper, and compared with the scale by the aid of compasses. The lines may be engraved upon a slate, and their value affixed, so that any object may be at once measured. We require of course a different scale for each power. Such scales are here made upon these pieces of gummed paper, and one of them may be affixed to every microscopical drawing. Thus the size of every object delineated may be at once ascertained, and the trouble of making individual measurements saved, while at the same time the inconvenience of a long description of the dimensions of various objects is avoided, than which nothing can be more tedious or less profitable to the reader.

The plan of appending scales to microscopical drawings is exceedingly convenient, and you may form some idea of its practical value by examining the drawings and lithographs upon the table.

Measuring the Angles of Crystals.—I have already adverted to the principal methods of measuring objects, but have not discussed the mode of ascertaining the value of the angles of microscopic crystals in the microscope. The simplest instrument for this purpose is the one which I now show you, which is a slight modification of *Schmidt's goniometer.* It

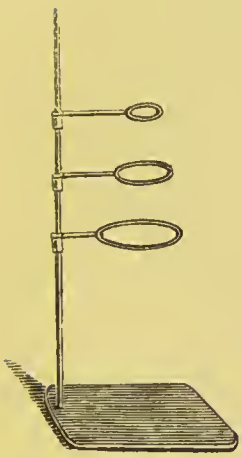
consists of a cobweb stretched across the field of an eye-piece, and capable of being moved by an arm which passes round an accurately graduated arc. The cobweb line is placed parallel to one face of the crystal, the circle being moved round until the bar stands at zero. The latter is then made to rotate until the cobweb is brought parallel with another face. The number of degrees through which the bar has passed marks the angle of the crystal. It is absolutely necessary that in taking this measurement the crystal should be perfectly flat, for otherwise a false angle will be obtained. Mr. Leeson has proposed a beautiful and much more perfect arrangement for measuring the angles of small crystals, which is described by Mr. Highley in the Fourth volume of the *Quarterly Journal of Microscopical Science*, page 77.

On Ascertaining the Magnifying Power of Object-glasses.— Permit me now to say a few words upon the magnifying power of the different lenses. Although the several object-glasses are termed one inch, one quarter of an inch, one eighth, &c., the magnifying power of each is not definite, and the quarters of some makers magnify many times more than those of others. It is well, therefore, that every observer should be able to ascertain for himself the magnifying power of his different glasses. Suppose I wish to know how much this French quarter magnifies. The one-thousandth of an inch micrometer is placed in the field, and the magnified image is thrown by means of the neutral tint glass reflector upon this scale, divided into inches and tenths of inches. The magnified one-thousandth of an inch covers about two-tenths of an inch, and consequently this glass magnifies about 200 diameters ; for if it covered one inch, the thousandth of an inch must have been magnified 1,000 times, but in this case it only corresponds to the one-fifth of an inch, and therefore the one-thousandth is magnified 200 times. For lower powers the one-hundredth of an inch scale may be employed. The manner of ascertaining the magnifying power is therefore exceedingly simple ; but it is very important for the observer to know the magnifying power of every lens, and he should ascertain this before he commences to make any observations.

Fig. 41.

Fig. 42.

PLATE XII.



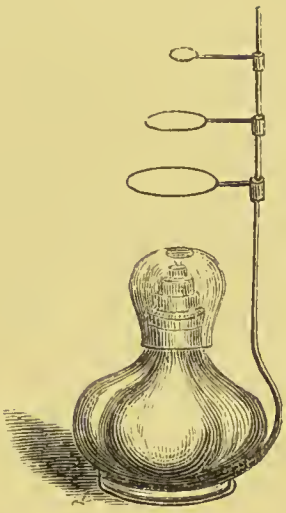


Fig. 43.

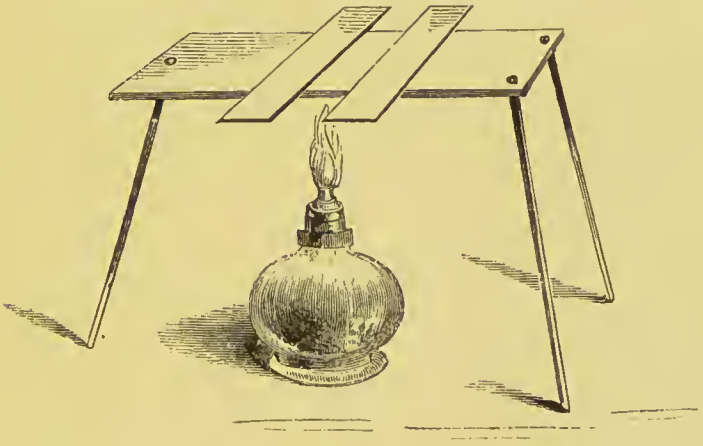
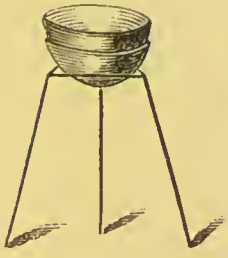


Fig. 45.

Fig. 44.



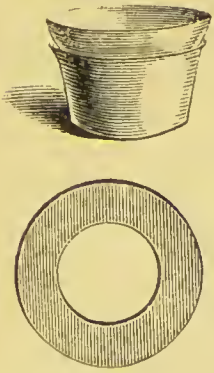


Fig. 46.



Fig. 41. Small retort-stand to support watch-glasses, &c. (p. 25).
Fig. 42. Spirit lamp with wire stand attached (p. 25).
Fig. 43. Brass plate for heating glass slides (p. 26).
Fig. 44. Porcelain basins arranged for a water bath (p. 26).
Fig. 45. Small copper bath, with ring to diminish aperture (p. 26).
Fig. 46. Tripod wire stand for supporting platinum basin containing matter for incineration (p. 25).

[To face page 24.]

LECTURE III.

Instruments required in microscopical research.—Spirit lamp.—Wire retort stands.—Water bath.—*Instruments for cutting thin sections of tissues.*—Scalpels.—Double-edged scalpel.—Double-bladed or Valentin's knife.—Scissors.—Needles.—Forceps.—*Apparatus used for examining objects in the microscope.*—Glass slides.—Thin glass.—Glass cells.—Watch glasses. *Cements.*—Gold size.—Shell-lac.—Brunswick black.—Marine glue.—Cement for attaching India-rubber or gutta percha to glass slides.—Canada balsam.—Gum.—French cement composed of lime and India-rubber.—*Preservative solutions.*—Spirit and water.—Glycerine.—Thwaites' fluid.—Solution of naptha and creosote.—Solution of naptha and water.—Solution of chromic acid.—Preservative gelatine.—Gelatine and glycerine.—Solution of chloride of calcium.—Arseniuretted hydrogen.—Nitrogen.

Gentlemen,—In the present lecture I propose to say a few words upon the apparatus necessary for microscopical investigation, and to show you the different instruments required by the microscopical observer.

In discussing this part of my subject I shall restrict myself to the consideration of that which is absolutely essential, and I shall not attempt to describe minutely different pieces of apparatus which various observers have proposed for special investigations.

Spirit Lamp.—The spirit lamp may be made of brass, tin, or glass fitted with a ground glass cap. It may be fitted with a stand for holding watch-glasses, like this one. Brass lamps, to which a small retort-stand is fitted, may also be purchased of the instrument makers.

Wire Retort Stands.—Simple wire stands, made like retort stands, which are fixed to a heavy leaden foot, will be found exceedingly useful little instruments to the microscopical observer. The rings can be readily raised or lowered at pleasure, and are well adapted to support light objects, such as glass slides over a lamp, test-tubes, flasks, and watch-glasses.

Tripods are made of thick iron wire, and are useful for

supporting several pieces of apparatus used in microscopical research.

Brass Plate.—The brass plate should be about six inches long by two broad, and about the thickness of thin millboard. It should be supported on three legs, of a convenient height for the spirit, or other, lamp to be placed underneath, or the brass plate may be supported on one of the rings adapted to Mr. Highley's lamp. It is used for heating glass slides, in order to fix on the glass cells with the aid of marine glue, for mounting objects in Canada balsam, and for other purposes, where a uniform degree of heat is required to be applied to glass, which is very liable to crack if exposed suddenly to the naked flame. These different pieces of apparatus have been placed on the table.

The Water Bath is of great use for drying objects previous to mounting them in Canada balsam. The object may be placed in a small porcelain basin or large watch-glass, or it may be simply laid upon a flat plate. The basin or plate is then placed over the vessel containing water to which heat may be applied. In order that vessels of different sizes may be heated upon the bath, it is convenient to have a few pieces of thin copper plate, with holes of different sizes cut in them, adapted for watch-glasses and small vessels. The advantage of drying by a steam heat consists in there being no danger of destroying the texture of the object by the application of too high a temperature. A water-bath may be very readily made by placing two porcelain basins one above the other, water being poured into the lower one. These may be supported upon a tripod or upon one of the rings over the spirit lamp.

Instruments for Cutting Thin Sections of Tissues.

Scalpels.—It will be convenient to have three or four ordinary dissecting knives or scalpels for general use. One should be strong for the purpose of cutting hard substances.

Double-edged Scalpel.—For cutting thin sections, a knife of the form of a very narrow lancet will be found useful, and where only sections of small dimensions are required, this will answer all the purposes of Valentin's knife, which I shall

PLATE XIII.

Fig. 47.

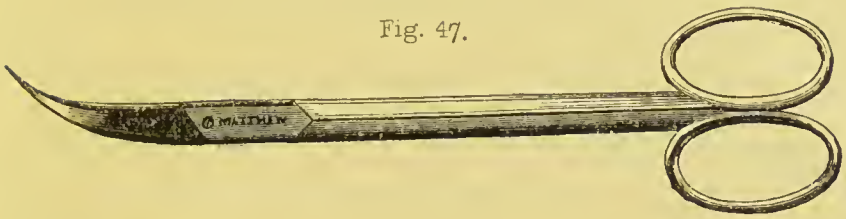


Fig. 48.

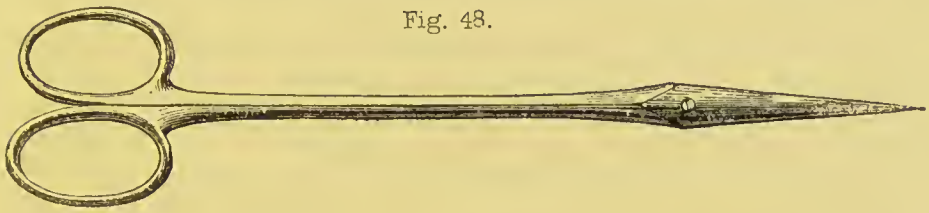


Fig. 49.



Fig 50.

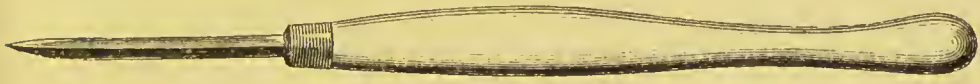


Fig. 51.



Fig. 52.

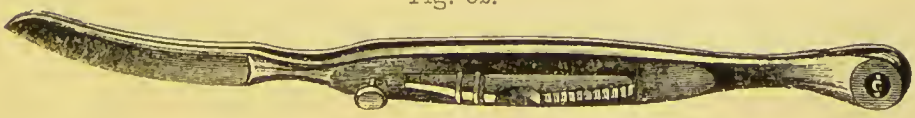


Fig. 53.



Fig. 47. Curved scissors, for cutting thin sections of tissues (p. 28).
Fig. 48. Fine straight scissors, for dissecting (p. 28). Fig. 49. Spring scissors, for making minute dissections (p. 28).
Fig. 50. Double-edged scalpel, for cutting thin sections (p. 26).
Fig. 51. Valentin's knife (p. 27). Fig. 52. Valentin's knife, as improved by Mr. Matthews (p. 27)
Fig. 53. Curved forceps, for dissecting.

[To face page 26.]

show you presently. In cases, however, where a section is wanted of considerable size, the latter instrument must be used. The double-edged scalpel should be very thin. When employed for making a section, after cutting a clean surface, the point is made to perforate the surface, and carried along at a proper depth, so as to cut its way out. The width of the section may then be increased by carrying the knife from side to side.

Double-bladed or Valentin's Knife.—This instrument is of the greatest value in making thin sections of soft tissues, but care is required to keep it in good order. It is soon made blunt if used for cutting fibrous or cartilaginous textures. By its aid very beautiful sections of the kidney, liver, and other soft glandular organs may be obtained with the greatest facility. The blades should always be dipped in water just before use, for, if wet, the operation of cutting is much facilitated, and the section more easily removed from between the blades. Immediately after use the blades should be washed in water, and dried with a soft cloth, or piece of wash-leather. If a drop of water gets into the upper part of the knife where the blades meet, the screw must be taken out, and each blade cleaned separately. With care in cleaning it, the knife may be kept in use a long time.

There are two forms of Valentin's knife ; in one the blades are sharp on both edges and of a lancet-shape, and in the other, which I much prefer, they are sharp at the point and wide at the base, so that the cutting edge slants downwards from the point, and they only cut on one side. The best form of Valentin's knife that I have used is that which I now show you. This has lately been made by Mr. Matthews. The blades of this knife can be completely separated from each other and easily cleaned. Moreover the distance between the blades is regulated by this little screw, which is a most convenient arrangement.

Scissors are useful instruments for cutting small thin sections of different tissues. The most convenient form for this purpose is one in which the blades are curved as in this instrument. When only very small portions of a tissue are required for examination, they will be more readily removed with the scissors than with any other instrument. Several

pair of scissors are required for microscopical purposes. Besides the ordinary form used for dissection, and a small pair, with curved blades, a pair of very delicate scissors, with blunt points, such as are employed for the dissection of insects, will be found of use. Here is a form of spring scissors, somewhat resembling the microtome, which I have been much in the habit of using, and which is adapted more especially for dissecting the nervous systems of insects, for following out the delicate ramifications of nerves and other minute dissections.

I shall describe the different pieces of apparatus required for injection in my seventh lecture, which will be exclusively devoted to this subject.

Needles of various sizes, form very useful instruments to the microscopical observer. They are employed for making minute dissections; for tearing or unravelling various tissues, in order to display their elementary structure, and for separating any minute object from refuse or extraneous matter, previous to its being mounted. Very thin needles are useful for separating substances under the field of the microscope. Needles which have been flattened at the points, and subsequently hardened, tempered, and sharpened on the two edges make capital knives for very delicate work, or the pins used by the surgeons and termed *harelip pins* may be used with advantage. They may be inserted in a small wood stick, or held in the handle of a crotchet needle. Mr. Matthews has lately made some needles with cutting edges, which are very useful for making minute dissections.

Forceps.—A pair of thin brass forceps will be found convenient for applying the thin glass cover, after the preparation has been placed upon a slide or in a cell. A pair of dissecting forceps are also required by the microscopist. One pair should be strong with straight limbs, the other pair should be small, with thin curved blades, terminated with slightly rounded points, of the pattern which I now show you.

Wooden forceps made of box-wood, with broad ends, are convenient for holding the glass slides when hot, for if held with cold metal forceps, they often crack. The same object may be gained more simply by fastening to the limbs of an ordinary pair of forceps flat pieces of cork.

Apparatus Used for Examining Objects in the Microscope.

Plate Glass Slides, the edges of which are ground and polished, may be obtained ready for use at six shillings per gross, or they may be easily cut out with the diamond, and the edges ground on the grinding-slab. The slides now in common use in this country are three inches in length and one in breadth, and I cannot too strongly recommend you to employ slides of this size only, for microscopical purposes. They should always be made of plate-glass, and pieces as clear as possible should be selected.

Thin Glass.—An object placed for examination upon a glass slide is always protected with a piece of thin glass before it is placed upon the stage of the microscope. Thin glass now used for microscopical purposes is called cylinder glass, and is manufactured at Birmingham. It may be obtained of different degrees of thickness. Thin glass in sheets should be kept in fine sawdust, as it is very readily broken, in consequence of being imperfectly annealed. When cut up in small pieces, it should be kept in a little box, with a little powdered starch, which prevents the pieces being broken. For cutting the thin glass an instrument termed a *writing diamond* is employed, and this is also used by some observers for writing the name of the preparation upon the glass slide. As a general rule, however, I think it better to write the name of the specimen upon a small label which can be gummed to the glass.

Glass cells I shall have to describe fully in my next lecture, so that it will not be necessary for me to detain you by alluding to their various forms, and the purposes for which they are employed, just now.

Watch glasses of various sizes should be kept by every observer, as they are convenient for many purposes. They cost about a shilling per dozen, and may be obtained of the watch-makers. The lunette glasses are useful for examining substances in fluids with low powers, as in these we are enabled to obtain a considerable extent of fluid of nearly uniform depth.

Cements.

The chief cements employed in microscopical work, are gold size, sealing-wax varnish, solution of shell-lac, solution of asphalt, marine glue, Canada balsam, gum, and a French cement composed of lime and India-rubber. These cements are used for fixing the glass cell on the glass slide, for fixing the cover upon the preparation after it has been properly placed in the cell, and for other purposes. The liquid cements should be kept in very wide-mouthed bottles, or in a capped bottle.

Gold Size is prepared by melting together gum animi, boiled linseed oil, red lead, litharge, sulphate of zinc, and turpentine. Gold size adapted for microscopical purposes may be also prepared as follows:—25 parts of linseed oil are to be boiled with one part of red lead, and a third part as much umber, for three hours. The clear fluid is to be poured off and mixed with equal parts of white lead and yellow ochre, which have been previously well pounded. This is to be added in small successive portions, and well mixed; the whole is then again to be well boiled, and the clear fluid poured off for use. In this country it may be obtained at any varnish makers.

Sealing-wax varnish is easily made by dissolving the best sealing wax of any colour which may be desired, in tolerably strong alcohol. This cement is, however, apt to dry rather brittle, and should not, therefore, be used in cases where it is of the greatest importance to keep the cell perfectly air-tight. It forms a good varnish for the last coat. Various colours may be kept according to taste.

Solution of Shell-lac is recommended by Mr. Ralphs for fixing down the thin glass cover. It is made by dissolving shell lac in spirits of wine. The shell-lac should be broken in small pieces, placed in a bottle with the spirit, and frequently shaken, until a thick solution is obtained. It dries rapidly, and, if put on in thin layers successively, forms a good cement. It is not acted upon by weak spirit.

Brunswick Black.—Solution of asphalt in turpentine commonly known by the name of Brunswick black, may be obtained at any oil-shop, and forms a most useful cement,

both for making very thin cells, and also for fixing on the thin glass covers. If a little solution of India-rubber in mineral naphtha be added to it, there is no danger of the cement cracking when dry ; a hint for which I have to thank my friend, Mr. Brooke. I have many preparations which have been cemented with Brunswick black which have been kept for nearly ten years. It is always desirable, however, to paint on a new layer from time to time, perhaps once in six months.

Common Brunswick black is made by melting one pound of asphaltum, and then adding half a pound of linseed oil, and a quart of oil of turpentine. The best Brunswick black is prepared by boiling together a quarter of a pound of foreign asphaltum, and four and a quarter ounces of linseed oil, which has been previously boiled with half an ounce of litharge until quite stringy ; the mass is then mixed with half a pint of oil of turpentine, or as much as may be required to make it of a proper consistence. It is often improved by being thickened with lamp black. It must be remembered that this cement is soluble in oil of turpentine.

Marine Glue.—This substance was, I believe, first used for microscopical purposes by Dr. Goadby, of Philadelphia. It is prepared by dissolving, separately, equal parts of shell-lac and India-rubber, in coal or mineral naphtha, and afterwards mixing the solutions thoroughly with the application of heat. It may be rendered thinner by the addition of more naphtha. Marine glue is readily dissolved by naphtha, ether, or solution of potash. It is preserved well in a tin box. I shall describe the manner of using marine glue and the different cements I have alluded to, when we next meet.

Cement for attaching Gutta Percha or India-rubber to the Glass Slides.—A cement for attaching cells of gutta percha or India-rubber to the glass slide may be made as follows :— According to Harting, gutta percha is to be cut into very small pieces and stirred, at a gentle heat, with fifteen parts of oil of turpentine ; the gritty, insoluble matter, which the gutta percha always contains, is to be separated by straining through linen cloth, and then one part of shell-lac is to be added to the solution, kept at a gentle heat, and occasionally stirred. The mixture is to be kept hot until a drop, when allowed to fall upon a cool surface, becomes tolerably hard.

When required for use, the mixture is to be heated, and a small quantity placed upon the slide upon which the cell is to be fixed ; the slide itself is then to be heated.

Canada Balsam is much employed by microscopical observers : formerly it was used for cementing cells together, but this is now effected more readily by the aid of marine glue.

Canada balsam is a thick viscid oleo-resin, which becomes softer upon the application of a gentle heat. If it be exposed to too high a temperature, the volatile oil is expelled, and a hard brittle resin remains behind. It is chiefly employed for mounting hard dense textures ; and, in consequence of its great power of penetrating textures, and highly-refractive properties, the structure of many substances, which cannot be distinguished in the ordinary mode of examination, is rendered manifest when immersed in this medium. Canada balsam should be preserved in a tin box such as this, care being taken to exclude the dust ; or in a bottle having a cap to it. The balsam should be kept very clean, otherwise preparations mounted in it will often be spoiled in consequence of the accidental introduction of foreign bodies. It has been frequently recommended that the oldest specimens of balsam should alone be employed for microscopical examination. By exposure to the air, the balsam becomes very thick, and unfit for use : it may be thinned by the addition of turpentine, but this should always be avoided as it renders the balsam liable to become streaky some time after the preparation has been mounted, and bubbles are often found in it. It is, however, always better to use balsam which has been kept in well-closed vessels.

Gum.—Thick gum-water will be found very useful for attaching labels to preparations, and also for fixing on the cover when preparations are mounted in the dry way. It is prepared by placing common gum-arabic in cold water, and keeping the bottle in a warm place until the solution has become sufficiently thick. It should always be strained before it is placed in the bottle for use.

Gum-water, thickened with powdered starch or whitening, will be found a very useful cement for fixing the glass cover on preparations mounted dry. When dry it forms a hard

white coating. The addition of a little arsenious acid will prevent the growth of mildew. Another very convenient solution is made by dissolving powdered gum in a weak solution of acetic acid.

French Cement composed of Lime and India-rubber.—The French cement composed of lime and India-rubber is very valuable for mounting all large microscopical preparations. The principal advantages are, that it never becomes perfectly hard, and thus permits considerable alteration to take place in the fluid contained in the cell without the entrance of air, and it adheres very intimately to glass, even if it be perfectly smooth and unground. Suppose this glass cover is to be affixed to this large cell containing fluid. A small piece of this cement is taken between the finger and thumb and carefully rolled round until it can be drawn out into a thread about the eighth or tenth of an inch in thickness. I apply this to the top of the cell, before introducing any fluid, and slightly press it down with the finger previously moistened. It adheres intimately. The preservative fluid with the preparation are now introduced and the cell filled with fluid which indeed is allowed to rise up slightly above its walls. The glass cover, rather smaller than the external dimensions of the cell, and slightly roughened at the edges, is to be gently breathed upon, and then one edge is applied to the cement in this way, so that it may be allowed to fall gradually upon the surface of the fluid which is now seen to wet each part of the cover successively, until it completely covers the cell, and a certain quantity of the superfluous fluid is pressed out. By the aid of any pointed instrument a very little cement is removed from one part, so that more fluid may escape as the cover is pressed down gently into the cement. The pressure must be removed very gradually or air of course will enter through the hole. A bubble of air entering in this manner may often be expelled again by pressure, or it may be driven out by forcing in more fluid through a very fine syringe at another part of the cell; but it is far better to prevent the entrance of air in the first instance. The edge of the glass cover being thoroughly imbedded in the cement, the small hole is to be carefully plugged up by a small piece of cement, and the cell allowed to stand perfectly still for a short

time, when it may be very gently wiped with a soft cloth. The edges of the cement may be smoothed by the application of a warm iron wire, and any superabundance removed with a sharp knife. A little Brunswick black or other liquid cement may be applied to the edges, for the purpose of giving the whole a neater appearance.

The cement is made as follows :—A certain quantity of India rubber scraps is carefully melted over a clear fire in a covered iron pot. They must not be permitted to catch light. When the mass is quite fluid, lime, in a perfectly fine powder, having been slacked by exposure to the air, is to be added by small quantities at a time, the mixture being well stirred. When moderately thick, it is removed from the fire and well beaten in a mortar and moulded in the hands until of the consistence of putty. It may be coloured by the addition of vermilion or other colouring matter. I have several preparations which have been placed in the creosote and naphtha solution in large cells, and they are now perfectly air-tight, although upwards of seven years have elapsed since they were first put up. The lime and India-rubber cement answers well for fixing on the glass tops of large preparation jars, and looks very neat ; but, if moderately strong spirit be used, a little air must be permitted to remain in the jar.

Preservative Solutions.

Spirit and Water.—Spirit and water form a well known and valuable medium for preserving anatomical preparations. In diluting spirit, distilled water only should be employed ; for if common water be mixed with spirit, a precipitation of some of the salts dissolved in it not unfrequently takes place, which renders the mixture turbid and unfit for use. Proof spirit will be strong enough for all general purposes, except for hardening portions of the brain or nervous system, when stronger spirit must be used. Two parts of rectified spirit, about sp. gr. ·837, mixed with one part of pure water, makes a mixture of sp. gr. 915-·920, which contains about 49 per cent. of real alcohol, and will therefore be about the strength of proof spirit. One part of alcohol, sixty over proof, to five parts of water, forms a mixture of a sufficient strength for

the preservation of many substances, and many microscopical specimens may be preserved in a solution more diluted than this. Within the last few years, the Government has permitted the use of methylated alcohol for various purposes in the arts, which pays no duty. This spirit answers well for preserving anatomical preparations, and is a great boon to all engaged in putting up large anatomical specimens. It may be obtained at the price of 5s. 6d. a gallon sixty degrees over proof of Messrs. Lightly and Simon, and of other distillers, in quantities of not less than ten gallons at a time.

In the first instance, application must be made to the Board of Inland Revenue, Somerset House, for permission to use the spirit, by letter, accompanied with the names of two respectable householders, who are willing to act as bond that the applicant only uses it for the purposes stated in his application. The probable quantity required annually must also be stated.

Glycerine.—A solution of glycerine adapted for preserving many structures is prepared by mixing equal parts of glycerine with camphor water. The latter prevents the tendency to mildew, or it may be mixed with naphtha and water, or with the creosote solution to be described presently. The degree of dilution will depend upon the nature of specimen. If the substance be at all opaque it will be necessary to employ strong glycerine. On the table are many preparations which have been preserved for several years in glycerine. Of the importance of glycerine, as a preservative fluid, I shall have to speak in my fourth lecture.

Glycerine is obtained by boiling oil with litharge. The oleate of lead remains as an insoluble plaster, while the glycerine is dissolved. It may be rendered free from lead by passing a current of sulphuretted hydrogen through it; and the clear solution, after filtration, may then be evaporated to the consistence of a syrup.

The glycerine which is now distilled by a patent process, and known as Price's glycerine, is much superior to the ordinary glycerine. It is perfectly colourless, free from all impurities, and of much greater density. The specific gravity of Price's patent glycerine is 1240, while the common is only 1196·6. The former costs about 6s., and the latter 2s. 6d. a pound.

Thwaites's Fluid.—This fluid has been much employed by Mr. Thwaites for preserving specimens of desmidiæ; but it is also applicable to the preservation of a vast number of animal substances.

It is made as follows:—

Water . . . 16 ounces.
Spirits of wine . . 1 ounce.
Creosote, sufficient to saturate the spirit.
Chalk, as much as may be necessary.

Mix the creosote and spirit, stir in the chalk with the aid of a pestle and mortar, and let the water be added gradually. Next add an equal quantity of water saturated with camphor. Allow the mixture to stand for a few days, and filter. In attempting to preserve large preparations in this fluid, I have found that it always became turbid, and therefore was led to try several modifications of it. The solution next to be described was found to answer very satisfactorily.

Water may also be impregnated with creosote by distillation. It should be remarked that M. Strausdurkheim has succeeded in preserving animal preparations in camphor water only.

Solution of Naphtha and Creosote.

Creosote . . . . 3 drachms.
Wood naphtha . . . 6 ounces.
Distilled water . . 64 ounces.
Chalk, as much as may be necessary.

Mix first the naphtha and creosote, then add as much prepared chalk as may be sufficient to form a smooth thick paste; afterwards add, very gradually, a small quantity of the water, which must be well mixed with the other ingredients in a mortar. Add two or three small lumps of camphor, and allow the mixture to stand in a lightly-covered vessel for a fortnight or three weeks, with occasional stirring. The almost-clear supernatant fluid may then be poured off and filtered if necessary. It should be kept in well-corked or stoppered bottles.

I have some large preparations which have been preserved in upwards of a pint of this fluid, for more than seven years, and the fluid is now perfectly clear and colourless. Some dissections of the nervous systems of insects have kept excellently; the nerves retain their white appearance, and have

not become at all brittle. Two or three morbid specimens are also in an excellent state of preservation, the colour being to a great extent preserved, and the soft character of the texture remaining. I have one preparation mounted in a large gutta percha cell, containing nearly a gallon of this fluid.

A solution of wood naphtha or pyroacetic spirit, in water has been recommended by Professor Quekett, and forms an excellent preservative solution, in the proportion of one part of the naphtha to ten of water. The solution is often a little cloudy, but may be made quite clear by filtration after the mixture has been allowed to stand still for some days.

One great advantage of these aqueous preservative solutions is that the natural appearance of the structure is very slightly altered. The solution, however, after a time, renders many of the more delicate structures more or less granular.

Solution of Chromic Acid.—A solution of chromic acid is well adapted for preserving many microscopical specimens. It is particularly useful for hardening portions of the nervous system previous to cutting thin sections. The solution is prepared by dissolving sufficient of the crystallized acid in distilled water, to render the liquid of a pale straw colour.

The crystallized acid may be prepared by decomposing 100 measures of a saturated solution of bichromate of potassa, by the addition of 120 to 150 measures of pure concentrated sulphuric acid. As the mixture becomes cool, crystals of chromic acid are deposited, which should be dried and well pressed on a porous tile, by which means the greater part of the sulphuric acid is removed, and the crystals obtained nearly pure.

Preservative Gelatine.—This substance was first employed for preserving microscopical textures by Mr. H. Deane, who gives the following directions for its preparation :—

Gelatine	1 ounce.
Honey	4 ounces.
Spirits of wine . .	½ ounce.
Creosote	6 drops.

Soak the gelatine in water until soft, and to it add the honey which has been previously raised to the boiling-point in another vessel. Next, let the mixture be boiled, and after it has cooled somewhat, the creosote dissolved in the spirits of

wine is to be added. Lastly, filter through thick flannel to clarify it.

When required for use, the bottle containing the mixture must be slightly warmed, and a drop placed on the preparation upon the glass slide, which should also be warmed a little Next, the glass cover, after having been breathed upon, is to laid on with the usual precautions, and the edges covered with a coating of the Brunswick black varnish. Care must be taken that the surface of the drop does not become dry before the application of the glass cover ; and the inclusion of air-bubbles must be carefully avoided.

Gelatine and Glycerine.—A mixture of gelatine and glycerine makes a very valuable medium for preserving different animal and vegetable structures.

The mixture may be made as follows :—A certain quantity of gelatine is allowed to soak for some time in cold water, until it swells up and becomes soft. It is then placed in a glass vessel and melted by the heat of warm water. When fluid, an equal quantity of glycerine is added and well mixed with it. The whole may then be strained through coarse flannel. This mixture may be kept for any length of time, and a very slight heat is sufficient to render it perfectly fluid.

Goadby's Solution.—

Bay salt . . .	4 ounces.
Alum	2 ounces.
Corrosive sublimate .	4 grains.
Boiling water . .	4 pints.

Mix and filter. This solution for most purposes may be diluted with an equal bulk of water. For preserving delicate preparations it should be even still more dilute. Goadby's solution is very valuable for preserving many anatomical specimens, but as it tends to render tissues hard and opaque, it is not adapted for the preservation of many structures which are to be examined in the microscope.

Burnett's Solution is a powerful antiseptic but not adapted for the preservation of microscopical specimens.

Other Saline Solutions.—Many other saline solutions have been employed by different observers. Of these, a saturated aqueous solution of chloride of calcium, free from iron, has been much recommended for preserving specimens of bone,

hair, teeth, and other hard structures, as well as many vegetable tissues. A solution of chloride of calcium has been used by Professor Schröder Van der Kolk, of Utrecht, for keeping sections of the spinal cord and preparations of nerves. Many of these, through the kindness of my friend, I have had an opportunity of seeing and can testify to their excellence.

A solution of *alum* in the proportion of one part of alum to sixteen of water has been found to answer pretty well for some substances. Gannal's solution, which consists of one part of *acetate of alumina* dissolved in ten parts of water; solutions of *common salt* (one part to five of water, with a little camphor), *corrosive sublimate*, *persulphate of iron*, *sulphate of zinc*, and solutions of several other salts, have been recommended as preservative solutions, but although adapted for the preservation of animal substances, they cannot be employed for microscopical specimens, in consequence of their tendency to render the textures very opaque and granular.

Arsenious Acid has been much recommended, and my friend Dr. Andrew Clarke has preserved many beautiful specimens of lung tissue and other structures in an aqueous solution of this substance.

Arseniuretted hydrogen gas has also been recommended for the preservation of animal substances, but it is not adapted for microscopical preparations. Dr. Richardson has lately kept animal substances from decomposition by immersing them in an atmosphere of *nitrogen*, which is prepared by placing a piece of phosphorous in a stone jar containing common air, and provided with an air-tight cover. The oxygen is soon exhausted, and no decomposition can take place.

I shall have to allude to the method of using these preservative solutions when I describe the mode of examining textures under the microscope, and preserving them as permanent objects. Most of the preservative solutions which I have described may be obtained of Mr. Matthews, Portugal Street.

LECTURE IV.

On the methods of making cells for microscopical preparations.—Cells for preserving microscopical specimens.—Cells for dry objects.—Brunswick black cell.—Cells made of tinfoil and marine glue.—Of cutting and grinding glass.—Of cutting the thin glass.—Stone or pewter slab for grinding glass.—Cementing glass together with marine glue.—Cleaning off superfluous glue.—Cells made of thin glass.—Simple methods of perforating the thin glass.—Deeper glass cells.—Small deep glass cells for injections—Built glass cells.—Deep glass cells made by bending a strip of glass in the blowpipe flame.—Moulded glass cells.—Troughs for examining zoophytes.—Animalcule cage.—Round cells proposed by Dr. Guy.

Cells for Preserving Microscopical Specimens.

Gentlemen,—All objects intended for microscopical observation should be protected with a cover of thin glass. This cover prevents the entrance of dust, and protects the object from exposure to the atmosphere. The fluid in which many objects are placed for examination would rise in vapor which would condense upon the object-glass, and give rise to great inconvenience were it not prevented from evaporating by a thin glass cover. If the thin glass, however, should press upon the object placed upon the glass slide, its distinctness will be impaired, or the structure may be entirely destroyed—an inconvenience which is prevented by placing some substance slightly thicker than the object with it between the glasses. If this entirely surround the object, a little cavity is made in which a specimen may be placed, and afterwards covered with thin glass without risk of injury from pressure. This is termed a cell.

Cells may be composed of various materials according to the thickness which may be necessary, or according to the nature of the substance to be placed within them.

For *dry objects* an efficient cell is readily made with a ring of paper or cardboard fixed with gum upon the glass slide, or a hole may be punched out of a piece of cardboard, wood, millboard, or gutta percha, or a vulcanized India-rubber ring

may be cemented to a slip of glass. Many other devices will occur to the mind of any one who wishes to make neat cells of this kind. If, however, the cell is intended to contain fluid, it must be made of some substance impervious to moisture.

Brunswick Black Cell.—A very thin cell may be made with a ring of Brunswick black or gold size which has been allowed to dry upon the glass slide.

The best form of Brunswick black cell is the circular one, which is so easily made by the aid of Mr. Shadbolt's excellent apparatus. The slide is placed on this little brass wheel which is made to revolve, while a brushful of Brunswick black is held at the proper distance from the centre, according to the diameter of the cell required.

Marine Glue Cells may be made according to the same plan. In order to make such a cell, a glass slide is warmed upon the brass plate (page 26) and when hot enough a small piece is allowed to melt upon the slide, being moved round and round in the position in which we wish to make the wall of the cell. When the glue has been allowed to cool, any superfluity may be removed from the slide with a sharp knife. On the table are several specimens of cells made in the manner just described.

Cells made of Tinfoil.—A piece of tinfoil may be cut out, so as to form a slightly thicker cell, and may be fixed upon the slide with marine glue.

Cutting and Grinding Glass.—In the manufacture of cells presently to be described, glass is required to be cut with a diamond and ground perfectly smooth at the edges. Moderately thick glass is cut with the ordinary glazier's diamond, but when we require to cut plate glass a larger diamond than that in ordinary use is necessary.

The *thin glass* is cut with the writing diamond, which makes a scratch sufficiently deep to permit of the glass being broken off very smoothly.

The *circles* of thin glass may be cut by carrying the diamond round the openings in these pieces of brass, of which we may have many different sizes so that circular pieces of thin glass of any required diameter, may be obtained.

Glass can be *ground* upon a perfectly *flat stone* with emery

powder or fine sand and a little water, or, instead of the stone, a flat *plate* composed of pewter may be used, as was recommended by Dr. Goadby. The emery after a time becomes imbedded in the pewter, and thus a very efficient surface for grinding is obtained.

The pewter plate may be cast in the form of flat circular disk which can be placed upon a pivot and made to revolve rapidly in a horizontal direction by means of a multiplying winch connected with it, an arrangement which is desirable when it is important to save labour as much as possible.

Cementing Glass together with Marine Glue.—The surface of glass to which a cement is to be applied should always be roughened by grinding, as the cement adheres much more intimately to a rough surface than to the polished glass.

Glass is cemented together with marine glue, and in making large built glass cells the edges are united by means of the same substance, which can now be readily obtained. Formerly gold size, Canada balsam, and other cements were employed, but these are all inferior to marine glue.

The manner of applying the marine glue to the glass has been already alluded to. The glass must always be warmed upon a plate, so that the heat may be applied gradually and equally. It must not be touched with cold fingers, but must be held with wooden forceps, or with ordinary forceps, the extremities of which have been protected with pieces of cork, in the manner described in my last lecture, p. 28.

When the pieces of glass of which the cell is to be composed are warm enough, a little glue cut into small pieces is allowed to melt in the position in which the glass is to be fixed. When it is melted, the glass is applied and pressed down upon a deal board, so as to squeeze out as much marine glue as possible.

Cleaning off Superfluous Glue.—While the slide is yet warm, much of the glue may be scraped off with an old knife and small chisel, after which a little *solution of potash* (the *liquor potassæ* of the shops) will soften the remainder. It may then be very readily removed with the aid of soap and water and a nail brush. Or the whole cell may be soaked in equal parts of liquor potassæ and water,—but we must bear in mind that if the cell be soaked for too long a time in strong solution of potash, there is danger of the glue between the glass, being

PLATE XIV.

Fig. 54.

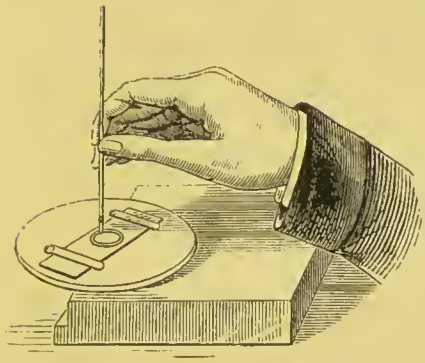


Fig. 55.

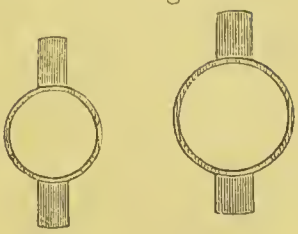


Fig. 56.

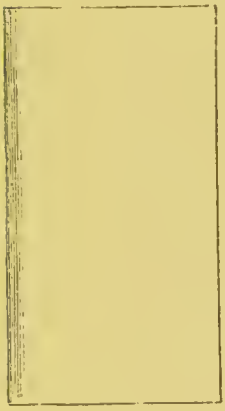


Fig. 57.



Fig. 58.

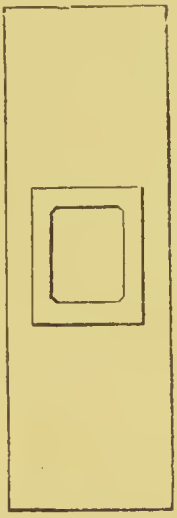


Fig. 59.

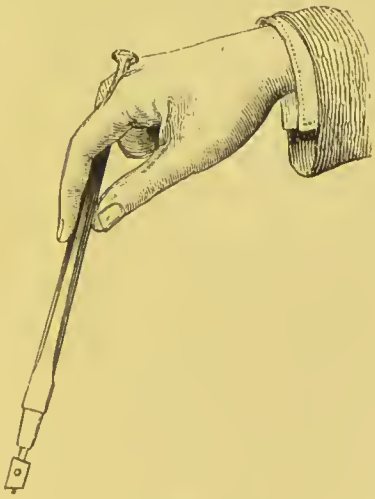


Fig. 60.

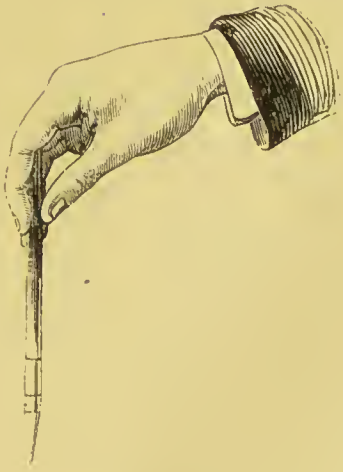


Fig. 54. Shadbolt's apparatus for making round cells of Brunswick black (p. 41).
Fig. 55. Flat brass rings for cutting circles of thin glass (p. 41).
Fig. 56. Plate glass stage for examining objects when immersed in acids or corrosive liquids.
Fig. 57. Large bradawl, for scraping away superfluous marine-glue in making cells (p. 42).
Fig. 58. Thin glass cell, for examining objects suspended in fluid (p. 43).
Fig. 59. To illustrate the manner in which the diamond is used for *cutting* thick glass (p. 41).
Fig. 60. Writing diamond for cutting thin glass (p. 41).

[To face page 42.]

softened. The potash must always be carefully washed away, to prevent the chance of the glue being softened after the cell is complete.

Cell made of Thin Glass.—The neatest and most perfect *shallow cell* is formed by making a hole of the required size in a piece of thin glass. This used to be effected as follows :—Many pieces of thin glass were glued together with marine glue, and when cold a hole was drilled through them all. Lastly they were separated from each other by heat, and cleaned with potash in the usual manner.

Simple Method of Perforating the Thin Glass.—Thin glass cells may, however, be readily made by every microscopist for himself, according to either of the following plans :—My friend, Dr. Frere, takes a small piece of thin glass, and with the writing diamond scratches a line corresponding to the piece of glass he wishes to remove, next a bradawl or other sharp instrument is placed in the centre of the space, the glass being laid upon a perfectly flat surface, such as thick plate glass. A sharp tap upon the bradawl with a light hammer causes it to perforate the glass, but the cracks made in it do not extend beyond the line marked with the diamond. The fragments of glass are then carefully removed piecemeal with a pair of fine forceps, and the cell is complete. In many cases, however, the cracks do pass beyond the line, and thus the chance of removing the fragments from the centre is much diminished.

The method which I have been in the habit of employing for some years is this : I cement a square or circle of thin glass with marine glue to one of the circular or quadrangular rings of glass used for making deep glass cells, and alluded to in page 44. The hole in the centre being the exact size of that required to be made in the thin glass. When the marine glue is cold, a file is forced through the centre of the thin glass, in this manner, and you may observe, that the cracks do not run across that part of the glass cemented by the marine glue. The edges may then be filed square, and the thin glass only requires to be warmed in order to remove it from the cell. It may now be fixed upon the slide at once, or cleaned with potash and kept with others until required to be made into a cell.

Deeper Glass Cells.—Supposing a cell a little deeper is

wanted, we may proceed in this manner :—a piece of plate glass of the proper thickness may be cut with the diamond to correspond with the outside of the cell, next, from each side of this piece of glass, a strip of the required width is to be removed, and from its ends, corresponding strips are to be cut off. The central portion is taken away, and the strips thus cut out are *inverted* upon the slide upon which they are to be fixed with marine glue, care being taken to mark them in the first instance, so that they may correspond properly with each other. The marine glue is allowed to run into all the corners. In this way a capital cell is very easily and quickly made. Cells of various sizes and depths can be manufactured upon this principle. The surface of the glass rim should be ground upon the stone, and the superfluous glue removed in the ordinary manner.

Small Deep Cells for Injections.—By drilling a hole in a piece of plate glass, by cutting off sections of various thickness from thick glass tubing, or from thick square glass bottles, or from vessels moulded for the purpose,—excellent cells of various dimensions, and admirably adapted for mounting injections and other purposes, are made ; but when the preparation is of considerable thickness, deeper cells than any of those to which I have alluded will be required. These may be made in glass, gutta percha, and some other substances. A *round* or *oval concavity* may be ground upon the surface of very thick piece of plate glass.

Built Glass Cells are those which are constructed by joining together at the edges and ends separate pieces of glass with marine glue or some other cement. The simplest form of built glass cell has been already described.

The cell which I now show you is made from thick plate glass, the edges of which have been ground perfectly flat before they were united with the marine glue. Dr. Goadby used to make many of these cells, which can be formed upon this principle of very large dimensions. They may be obtained of Mr. Dennis, of St. John's Street Road, who has succeeded in making cells in this manner large enough to hold several quarts of fluid. Many cells of this description may be seen in the Hunterian Museum of the Royal College of Surgeons. They may be constructed as follows :—A strip of plate glass is

PLATE XV.

Fig. 61.

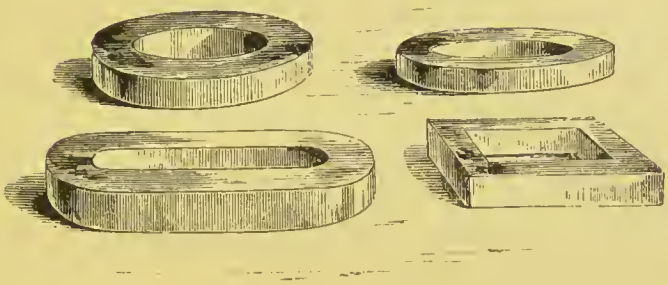


Fig. 62.

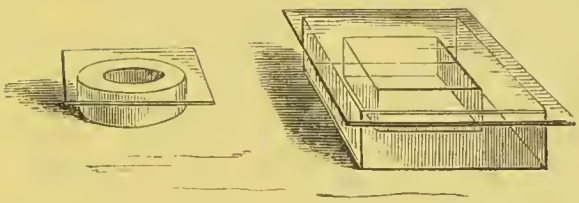


Fig 63.



Fig. 64.

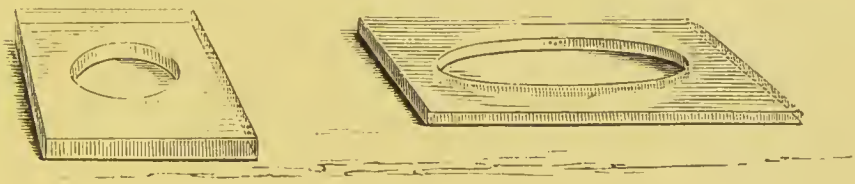


Fig. 65.

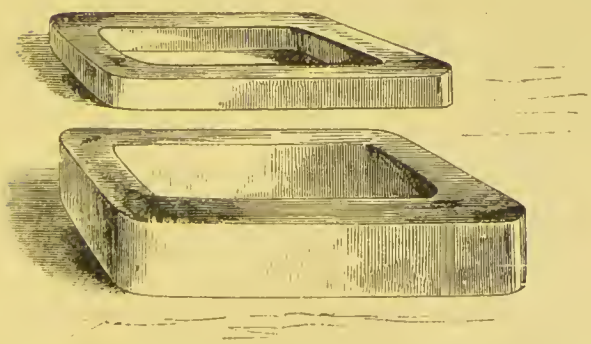


Fig. 66.

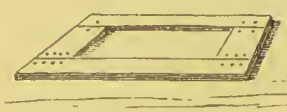


Fig. 61. Small cells for preserving injections and other thick preparations (p. 44).
Fig. 62. To illustrate the manner in which the thin glass may be perforated for making thin glass cells (p. 43.)
Fig 63. Shows the way in which the angles of a built glass cell are joined together (p. 45).
Fig. 64. Glass cells made by grinding out the centre of a piece of plate glass (p. 44).
Fig. 65. Large deep glass cells, for preserving opaque preparations (p. 44.)
Fig. 66. Illustrates a simple way of making a moderately thick glass cell (pp. 43, 44.)

[To face page 44.]

cut off, of the proper height for the sides of the cell. From this, two pieces are to be cut off the desired length of the sides, and two pieces for the ends. The flat surfaces of these are to be cemented with marine glue, and all the edges ground perfectly flat together. The ends are also to be very carefully ground square. They are then to be separated by heat and connected together at the corners in the proper position. When the four sides have been thus joined together, one surface is to be carefully ground flat, and then cemented to the plate glass bottom. The other side on which the cover is to be placed may be ground flat afterwards. In order to increase the strength of these cells and to diminish the chance of leakage, it is well to cement small pieces of glass in the corners, and narrow strips outside where the sides are attached to the glass slab.

These cells, of course, take some time to make, but they are exceedingly neat, and have but one serious drawback—a slight liability to leak, which is hardly to be wondered at when the number of the joinings is taken into consideration.

Deep Glass Cells made by bending a strip of glass in the blow pipe flame.—For some years past I have been in the habit of bending a long strip of glass in the blow-pipe flame, and cementing the extremities together in a similar manner whenever a cell of about half an inch in depth is wanted. The ordinary plate glass is very liable to crack as it becomes cool, but if *flatted flint glass* be employed the operation is simple enough. This glass, as well as the deep glass cells above referred to, may be obtained at Messrs. Powell's glass-works, Whitefriars. This cell has the disadvantage of not being perfectly clear. If flint glass could be flatted, ground, and polished like plate, it would be of much value to those who mount large objects in deep glass cells.

Moulded Glass Cells.—Of late years, moulded glass cells have been much employed for anatomical preparations, and the absence of joints renders them preferable to built glass cells. Large moulded cells are now made in Germany, the sides of which have been ground and polished, and thus a preparation can be seen within almost as clearly as if the sides were composed of plate glass. These cells can be obtained for a much lower price than the built cells, and are, of course, not so liable

to leak. They may be purchased at the Glass-works, Whitefriars.

Gutta Percha may be moulded in a wooden case and forms excellent cells where transparent sides are not required. I have several preparations which have been preserved for several years in large cells of this description.

Troughs for Examining Zoophytes.—These are deep but very narrow glass cells, the two surfaces consisting of very thin glass, so that the higher powers may be brought sufficiently close to the objects. The opening is above, so that the cells with living animals within may be placed upon the stage of the microscope, when the instrument is inclined, without any fluid escaping. It is convenient to have a glass partition in these troughs, by means of which objects may be placed in different parts of the cell. A convenient size is three inches long, an inch and a half deep, and a quarter of an inch in width.

Animalcule Cage.—Another very convenient form of cell is the one which I now show you. By means of its sliding cover a stratum of fluid of any required thickness can be obtained, and small living animals can be conveniently fixed in positions suitable for observation. For the examination of deposits in fluids this form of cell is also very convenient.

Round Cells.—My friend and colleague Dr. Guy has lately proposed a form of cell which possesses many advantages over those in common use. These are circular, and may be made of bone, metal, gutta percha, or glass, of various depths, and to suit transparent and opaque objects. Several forms are placed on the table. They are all of the same external diameter, and are made to fit into a rim of equal size in this flat plate of wood, or metal, which can be placed in the field of the microscope. A small cabinet will contain many more preparations mounted in this manner than on the ordinary slips of glass. Dr. Guy has had some circular labels printed for these cells upon which the names of the preparations may be written, and as these are of different colors the various microscopic objects can be readily classified.

I think I have now said all that is necessary with reference to the microscope and various pieces of accessory apparatus which are required by the observer. I have also shown you the

PLATE XVI.

Fig. 67.



Fig. 68.

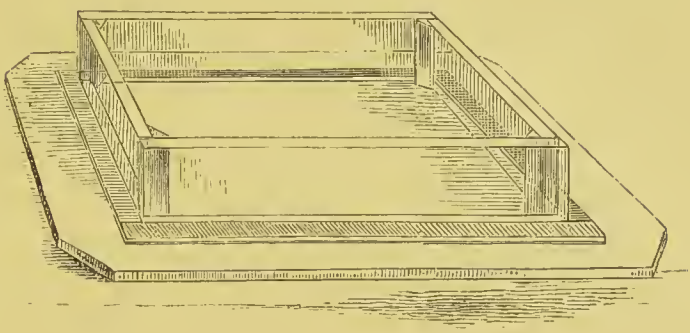


Fig. 69.

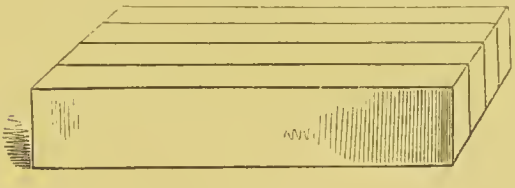


Fig. 70.

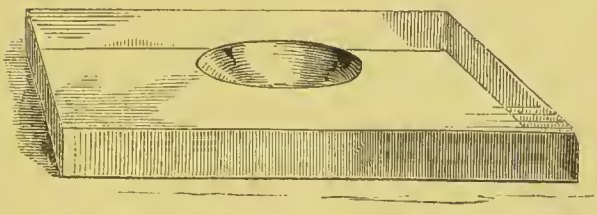


Fig. 71.

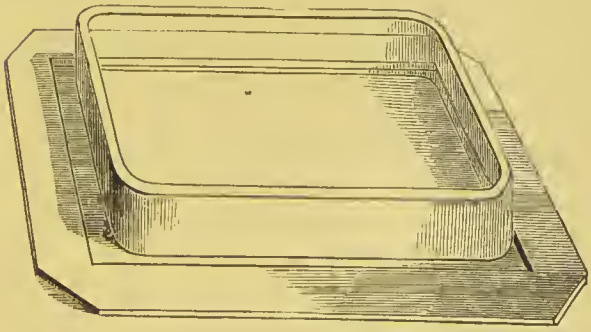


Fig. 67. To illustrate the manner in which cells of a peculiar shape may be made. The lower part is made of plate-glass, to which the tube is attached by gutta percha. This apparatus was made for examining the circulation in the branchiæ of a proteus. The smaller tubes were for the purpose of supplying the animal with fresh water.

Fig. 68. Large built glass cell (p. 44).

Fig. 69. Shows the manner in which the sides of built glass cells are cemented together in order to grind them perfectly flat (p. 44).

Fig. 70. Concave glass cell made by grinding out a cup-shaped cavity on the surface of a piece of very thick glass. It is afterwards polished (p. 44).

Fig. 71. Deep glass cell, made by bending a piece of glass in the blowpipe flame (p. 45).

[To face page 46.]

instruments necessary for ordinary microscopical research, and have described the different cements and preservative fluids employed. The plans at our disposal for the manufacture of cells which are necessary for the examination or preservation of objects have also been discussed. In my next lecture I shall have to draw your attention to the general characters of different tissues, and the methods by which their minute structure may be demonstrated in the microscope.

LECTURE V.

On examining objects in the microscope.—General considerations upon the structure of tissues.—Flesh or muscular tissue.—On demonstrating the anatomical peculiarities of tissues.—The anatomy of organs more easily demonstrated in the lower animals than in man and the higher animals.—Of the time after death when tissues should be examined.—Ciliary motion.—*Of preparing tissues for microscopical examination.*—Of making minute dissections.—Dissecting under the surface of fluid.—Tablets upon which dissections may be pinned out.—Of obtaining thin sections of different textures for mieroscopical examination.—Drying the tissue before cutting the section.—Hardening the tissue.—Horn.—Hair.—Making thin seetions of bone.—Teeth.—Sections of wood.—Of dissecting tissues under the microseope with the aid of the eompressorium.—*Of the importance of examining objects in various ways.*—Appearances of the same object in air, water, and Canada balsam by transmitted light, and under the influence of refleeted light and polarized light.

General Considerations upon the Structure of the Tissues.

Gentlemen,—The tissues of animals and plants for the most part are compound, and made up of several distinct elementary structures. For example, the smallest portion of this

flesh or muscular tissue, which I can remove with a knife or pair of scissors, is composed of several distinct structures. In the first place must be noticed the *proper substance* peculiar to muscular tissue, in which the characteristic contractile power of muscle resides. Secondly, at least in most cases, we find a tube composed of perfectly clear, *transparent, structureless membrane*, in which this contractile substance, or sarcous matter, is contained. Thirdly, there exists a certain quantity of *areolar tissue*, which connects together these elementary fibres ; and not unfrequently associated with this is a little *fatty* or *adipose tissue*. Fourthly, are *vessels*, lying between the elementary fibres just described, in which the blood circulates, for the supply of the tissue with its proper nutritive elements. In the fifth place we find nerve-fibres running in the same position as the vessels : and lastly, at least in relation with some of the fibres, are lymphatic vessels.

Thus, muscle is composed of several elementary structures, each having special anatomical peculiarities, and differing from the others in physical characters and chemical properties. Some of these structures refract light very highly ; others, only in a very slight degree. One may be greatly altered or even destroyed within a very short time after the muscle has been removed from the body, or by the action of plain water, while others resist decomposition for a great length of time. The characters of one may be demonstrated when the muscle is examined in water ; a second, when it is immersed in syrup or glycerine ; a third, when the specimen is mounted in Canada balsam ; while the arrangement of the delicate, transparent, capillary vessels cannot be satisfactorily made out unless a particular plan of preparation be adopted. Such is the nature of the textures in ordinary flesh which are demonstrable by the aid of the microscope. The chemist can detect a host of other compounds of whose presence the mere microscopist would ever remain unconscious of, for they are dissolved in the juices of the muscle, and therefore incapable of being detected by the eye alone.

The vast difference in the properties of the several textures above enumerated renders it absolutely impossible that all can be distinguished in one single specimen, for the circumstances which favor the exhibition of one structure will necessarily

PLATE XVII.

Fig. 72.



Fig. 73.



Fig. 74.

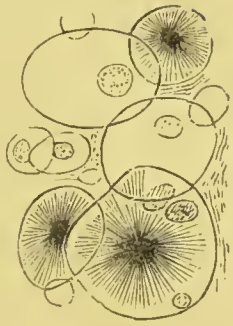


Fig. 76.



Fig. 75.



Fig. 77.



Fig. 72. Yellow fibrous tissue.
Fig. 73. White fibrous tissue (p. 48).
Fig. 74. Fatty tissue from the common newt (p. 48).
Fig. 75. Vessels of the gall bladder injected. A double injection of arteries and veins (p. 79).
Fig. 76. Tracheæ, or air tubes, from an insect (p. 104).
Fig. 77. Fibres of yellow elastic tissue.

[To face page 48.]

render another quite invisible. Hence, before we can hope to demonstrate satisfactorily the anatomical peculiarities of any one of these different textures we must become acquainted with its general properties, and must consider the mode of examination likely to be most efficient in rendering these distinct.

The walls of the smallest vessels are so thin and transparent that it is necessary to fill them with some coloured fluid or material more or less opaque, if we wish to see the mode of arrangement of the vascular network ; while this same process, as ordinarily followed out, precludes the possibility of tracing the finer ramifications of the nerves, and other elementary tissues are hidden and compressed by the distended vessels. To demonstrate the nerves, all the other structures must be rendered as transparent as possible, by the application of a chemical agent, or by immersing the specimen in a highly refracting fluid. In order to show the membrane in which the *sarcous tissue* is contained, the latter must be ruptured within it in a perfectly fresh specimen, or it must be separated from it by pressure. By one plan of proceeding it may be shown that the elementary fibre of muscle may be divided longitudinally into a number of minute *fibrillæ*, arranged parallel to each other ; while under other circumstances it can be separated transversely into a pile of *small disks*, or into a number of small elementary particles of definite form and size, by the connexion of which to the contiguous particles, the *fibrillæ*, or the *disks*, are produced, according as the particles adhere to each other most intimately by their sides or by their extremities. I might adduce many other instances of the necessity of studying the general character of tissues before any minute examination of the individual structures is attempted. In the present course it will only be possible for me to allude very cursorily to special processes adapted for the demonstration of particular textures.

On Demonstrating the Anatomical Peculiarities of Tissues.— Now, some observers who have not sufficiently considered the different characters of the elementary structures of which most of the organs of the body are composed, have strongly objected to what they term *methods of preparation*, considering that by these processes, structures are even *formed* which have no real existence in the natural state of the part. For this

view there is some reason. Doubtless, from the examination of a dead tissue we can form but an imperfect conception of the beauty of its elementary parts, and their wonderful adaptation to the office they are designed to perform in the animal economy; neither can we form an idea of the changes it undergoes during its vital activity; and we must remember that there is no known fluid in which we can immerse a specimen for examination, which possesses the precise characters of that which bathes the tissue during its lifetime. Serum may, perhaps, be the nearest approach to such a fluid, but there is every reason to believe that this differs from the fluid surrounding the primitive particles almost as much as some artificial media which have been proved by experience to give very satisfactory results. Objectors to the preparation of tissues have not satisfactorily proved that many of the structures which we see after death have a precisely similar appearance during life, and it is more than probable that many of the more delicate tissues have never been seen by any one in the condition in which they exist during life. I believe that the amount of opacity which is absolutely necessary for seeing some of these is quite inconsistent with their vitality, and is the result of a change which has never been fully appreciated, though, perhaps, some idea of its nature may be formed by considering the characters of fibrin in living blood, and fibrin removed from the organism and coagulated, or those of albumen dissolved in the serum, coagulated but transparent in many of the tissues, coagulated and opaque after the addition of different reagents.

From what I have just observed it must be evident, that the clear demonstration of the structure of any individual organ of the body is a somewhat difficult matter, and requires a considerable amount of knowledge of the chemical and physical characters of the tissues, as well as patient investigation and earnest study, which will alone enable us to make artificially a fluid which shall possess the most important characters of that which surrounds the tissue during its life.

The Anatomy of Organs more easily Demonstrated in the Lower Animals than in Man and the Higher Animals.—In consequence of the great complexity of the structure of many of the tissues of the higher animals their rapid change after

PLATE XVIII.

Fig. 78.



Fig. 79.

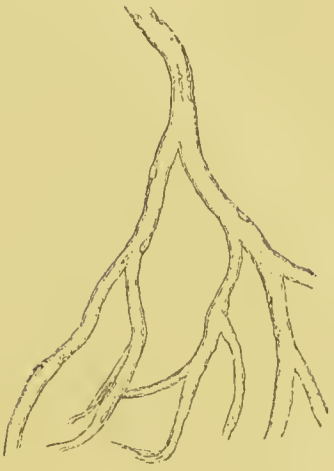
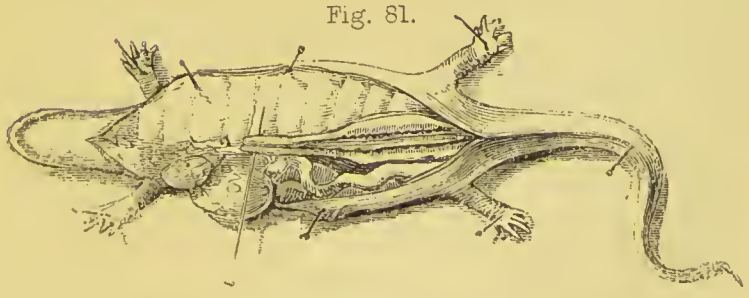


Fig. 80.



Fig. 81.



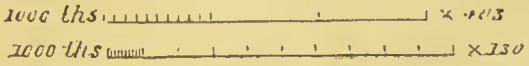


Fig. 78. Fatty tissue shewing fat vesicles, the crystalline fat has separated from the oily fat × 130.
Fig. 79. Small vessel dividing into capillaries × 215.
Fig. 80. Capillary vessels. *a*. Showing nuclei on walls. *b*. Fragment of a capillary vessel with membranes, *c*, attached. *d*. Another fragment flattened between the glasses (p. 49).
Fig. 81. Newt dissected to display the thin part of the kidneys under which a needle, *a*, has been placed (p. 51).

[To face page 50.]

being removed from the body, and their extreme delicacy, anatomists have long been in the habit of resorting to the examination of textures in the lower forms of animal life for obtaining an insight into the structure of parallel tissues in the higher, and with considerable success. I can adduce no better example of the great value of such an appeal to the simpler forms of animal life than occurs in the case of the *kidney*. In animals generally, this gland consists essentially of a vast number of long and highly tortuous tubes—which in the higher members of the class are packed so closely together that they form a firm and very compact organ, the general characters of which are familiar to all—and of vessels bearing a particular relation to these tubes. In such a kidney it is impossible, under ordinary circumstances, to follow a tube for any very great length, as you will be convinced if you look at the specimen in this microscope ; but in the lower animals the kidney is less compact, and the several tubes are not so intimately connected together. Indeed, in many of them the kidney is prolonged into a thin, transparent, almost thread-like organ, which extends into the thoracic portion of the animal. In this situation in the common *newt* or *eft* (Triton or Lissotriton) we have, so to say, a natural dissection of the elements of the gland structure, and we may *demonstrate* an arrangement, the existence of which we can only *infer* by an examination of thin sections of the compact kidney of mammalian animals. Single tubes, with the structures connected with them, may be traced throughout their entire length, and are quite separated from each other. I need hardly observe, that it would be vain to attempt to make such a dissection artificially. Many other instances of the value of this kind of investigation might be adduced of equal interest and importance, but instead of occupying time in this manner, permit me most strongly to urge upon all those who are likely to prosecute researches upon the characters of any particular tissue or organ, to investigate carefully its nature in the different members of the creation, and especially in the lowest forms in which its existence has been proved,—for there we may be sure to find it in its simplest condition, and our mind will be better able to appreciate the exact meaning of the structures which are superadded, and the more elaborate anatomical detail which we

shall meet with in the higher animals, than if we commenced our researches upon the most perfect examples of the structure.

Of the Time after Death when Tissues should be Examined.—I must also make a few remarks upon the time when the examination may be carried on with the best chance of success. Some animal tissues require to be examined very soon after death, or their characteristic peculiarities are lost. Upon certain surfaces in the higher animals, and to a greater extent in the lower classes, we find that the cells which generally form the outer coating to more delicate structures beneath, are provided with very active vibratile processes, or *cilia*, which by their movements create currents often of some considerable power. These movements are sometimes required to promote the rapid removal of foreign bodies which would injure delicate surfaces if they came in absolute contact with them, or for promoting a constant change in the fluid medium by which the animal is surrounded. The importance of cilia in effecting the latter object is seen in the greater number of shell fish, which are stationary throughout life, and are not provided with an apparatus for promoting a continual change of the fluid which bathes the surface of their respiratory organs. In these animals this great object is entirely effected by the agency of these small cilia.

Ciliary Motion endures for a longer or shorter period after death, and is entirely independent of the nervous system. In the active birds it ceases very soon, but in the more slowly-nourished, cold-blooded animals it often lasts for many days after death.

Ciliated epithilium can always be seen in the mucus scraped from the tongue of the frog, in the kidneys of this animal and of the newt, and on the gills of the mussel and oyster, of which a small piece may be removed with a pair of scissors. It is important to moisten ciliated epithilium with a little serum of the animal, as water soon puts a stop to the movements.

Of Preparing Tissues for Microscopical Examination.

Of Making Minute Dissections.—I shall now briefly describe the mode of dissecting delicate textures, and of removing small portions for microscopical examination.

Minute dissections are usually carried on under the surface

PLATE XIX.

Fig. 82.

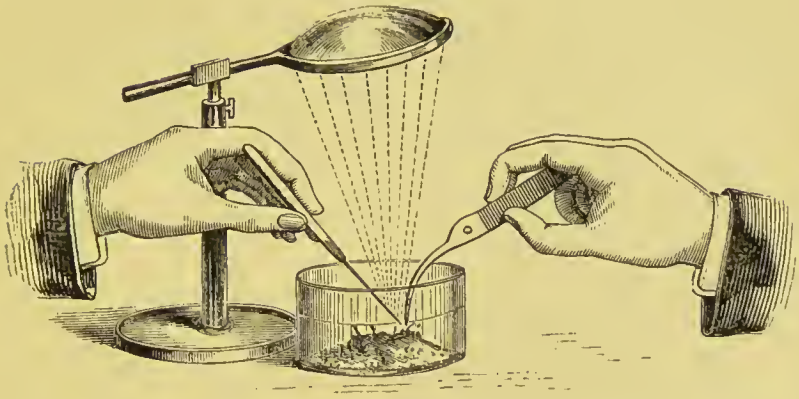


Fig. 84.

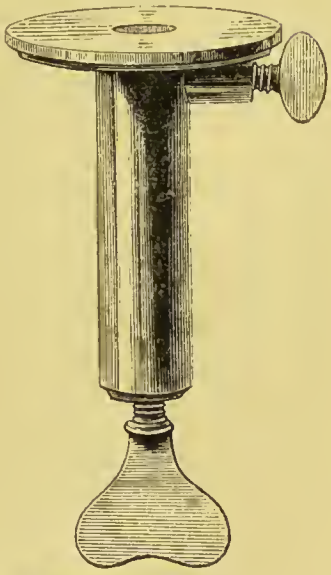


Fig. 83.



Fig. 85.

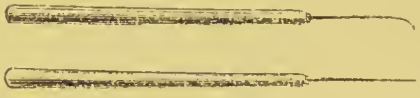


Fig. 86.

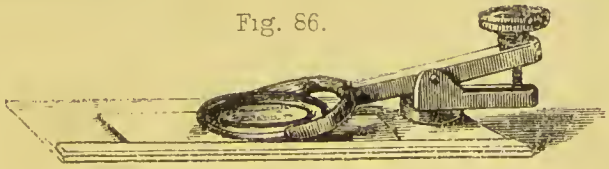


Fig. 87.



Fig. 82. Arrangement for dissecting objects under water. The bull's-eye condenser is larger than those represented in figs. 20, 21 (p. 52). It may be obtained of Mr. Matthews.
Fig. 83. Loaded cork, upon which objects for dissection may be pinned out (p. 53).
Fig. 85. Needles for dissecting (p. 28).
Fig. 84. Instrument for cutting thin sections of wood, &c. (p. 56).
Fig. 86. Compressorium, for pressing or tearing-up tissues under the microscope (p. 56).
Fig. 87. Saw for cutting thin sections of bone (p. 55).

[To face page 52.]

of fluid with the aid of small scissors, needles or small knives, and forceps. If the preparation has been preserved in spirit or other solution, it must be dissected in the same fluid, but in ordinary cases clear water may be used. The microscopist should be provided with a few small dishes, varying in size, and about an inch or more in depth. The large built cells make very good troughs for dissecting in, but small circular vessels are made on purpose.

Loaded Corks.—The object to be dissected is attached to a loaded cork by small pins. We may take a piece of flat cork rather smaller than the cell, and then cut a piece of sheet lead somewhat larger than the cork. The edges of the lead are then folded over the cork and beaten down slightly with a hammer, and may afterwards be filed with a rough file.

The object being fixed upon the cork and placed in the cell, fluid is poured in until it just covers the surface of the object. A strong light is then condensed upon it by means of a large bull's eye condenser, or by a large globe full of water. With a strong light, magnifying glasses are not required ; and I have always found that delicate dissections could be made with the greatest facility without the aid of a dissecting microscope, provided a strong light was condensed upon the object. Occasional examination of the dissection with a lens of low power is advantageous ; but if a lens be employed during the dissection there is great danger of accidentally injuring the specimen, as it is impossible to judge of the distance which the needle point may be beneath the surface of the fluid. Minute branches of nerves or vessels may in this way be followed out, and small pieces of the different tissues into which they can be traced may be removed for microscopical examination with a pair of fine scissors. Membranes may be dissected from the structures upon which they lie in a similar manner. By this plan the nervous system of the smallest insects can be very readily dissected.

Tablets upon which Dissections may be Pinned out.—Many preparations require to be arranged in a particular position previous to being mounted as permanent objects. *Slabs of wax* are usually employed by anatomists for this purpose, but when transparency is required the dissections may be attached by threads to thin plates of *mica*.

I have found that the best slabs may be made of a mixture of *wax* and *gutta percha*, in the proportion of one part of the former to two of the latter. The ingredients are to be melted in an iron pot, over a clear fire, and well stirred. When quite fluid, the mass may be poured upon a flat slab and allowed to cool. Thin cakes of about the eighth of an inch in thickness are thus obtained, and they can easily be cut with a knife to fit the cells intended for the preparation. Pins or small pieces of silver wire may be inserted into these slabs, and will adhere firmly although the slabs are very thin.

Of obtaining Thin Sections of different Textures for Microscopical Examination.—The general modes of obtaining thin sections of soft tissues was described in my third lecture, and it is only necessary for me now to illustrate the remarks I then made by making sections of some of the tissues on the table.

It is scarcely necessary to observe that such different textures as muscular fibre and gland structures, and other soft tissues require a different process for cutting them, to that which is applicable for cutting thin slices of such tissues as hair, horn, bone, or teeth.

Where thin sections of no very great extent of tissue are required they may be obtained by scissors, by the ordinary scalpel, by the double-edged knife, or by Valentin's knife, in the manner described in page 27. Whenever a thin section of a tissue is made, the instrument employed must be thoroughly wetted with water, and the section, after its removal, should be carefully washed, by agitating it in water, or by directing a stream of water upon it, from the wash-bottle (page 71). This washing is absolutely necessary to remove from the surface of the section particles of debris, which would render the appearances indistinct, and interfere with the clearness of the specimen when it was subjected to examination in the microscope.

Drying the Tissue before Cutting the Section.—There are, however, many tissues, of which sections cannot be obtained in this simple manner,—thus it is almost impossible to cut sections of soft membranous textures perpendicular to the surface, sufficiently thin for examination. In such cases, it is advisable to pin the texture out upon a board when perfectly fresh, and expose it to the atmosphere until quite dry. Thin

sections may then be cut very easily, and upon being moistened with water will resume their recent appearance.

Hardening the Tissue.—Other textures, again, require special treatment in order to render them sufficiently hard to enable us to cut thin sections. Some require boiling for this purpose, others soaking in alcohol, or chromic acid, or in syrup, while not a few require special modes of treatment, which are applicable to them alone.

Horn.—Thin sections of horn and textures of this description may be cut with a sharp strong knife.

Hair.—There are many ways of obtaining thin sections of hair. Thus a number of hairs, may be united together by a little gum, so as to form when dry, a firm hard mass. Thin sections of this can readily be made, with a sharp knife, and the individual pieces may be separated from each other, by the application of a drop of water. These may be mounted in fluid, or dried and preserved in Canada balsam.

Or the hairs may be placed between two pieces of cardboard, or between two flat pieces of cork, and when tightly pressed in a vice, thin sections of the hair, including the cardboard and cork, can be obtained with a sharp knife. For cutting thin transverse sections of hair, my friend Professor Weber of Leipzig, recommends a very simple expedient. He suggests that the beard should be shaved very closely, and then after a few hours shaved again. In this way excessively thin sections of hair in great numbers may be obtained.

Making Thin Sections of Bone.—For obtaining thin sections of bone, a totally different process is requisite. In the first place, a section as thin as possible is removed from the bone with the aid of a thin sharp saw. This may be made somewhat thinner by a file, and afterwards ground down to the required degree of tenuity upon a hone. The best stones for this purpose are the Turkey stones, which have been ground perfectly flat. The section may be kept in contact with the stone by the pressure of the thumb, or with a piece of cork, or by the finger, or lastly it may be rubbed between two hones, a proceeding which saves much time.

It is to be ground down with the aid of a little water, and when sufficiently thin it may be subjected to examination in the microscope. It will, however, now be found, that the

beauty of the tissue is completely obscured, owing to the number of scratches upon its surface. These may be removed by rubbing the section upon a dry hone, and afterwards upon a piece of plate glass. After the piece of bone has been properly polished, no lines will be seen upon it, when it is examined in the microscope.

Teeth.—Sections of teeth cannot be advantageously prepared in this manner, owing to the very brittle nature of the enamel. The better way is to grind the tooth down at a dentist's lathe until a section sufficiently thin be obtained.

Sections of shells of many of the lower animals, and the hard shells and stones of fruit may be made in a similar manner.

Sections of wood may be made with the aid of a little instrument which I now shew you. A piece of wood, after having been allowed to soak for some time in water, is placed in this hole, and kept in its position by the side screw. Upon turning the lower screw the wood is forced above the brass plate. A clean section is now made with a sharp strong knife or razor. By turning the screw beneath, very slightly, the wood is forced above the surface of the brass plate, and thus a section of any required thickness may be obtained.

Of Dissecting Tissues under the Microscope with the aid of the Compressorium.—In many cases the observer wishes to dissect an extremely delicate structure under the microscope, in which case much information can often be acquired with reference to the relation existing between the structural elements of the tissue. These objects may be gained by means of a little instrument termed a *compressorium* which consists simply of a convenient arrangement by which pressure can be applied to an object while under examination. This pressure being applied gradually, the texture becomes frayed out as it were, and particular structures can often be teazed out from a tissue, and demonstrated more distinctly than by any other method.

The structure of the compressorium is very simple. This one consists of a thick brass plate with a hole in the centre to admit the light. On one side of this is situated the fulcrum of a lever, the short end of which acts upon a circular ring carrying the thin glass to cover the preparation, while to the longer arm is attached a screw, which by being turned causes

the thin glass to be pressed tightly upon the object placed upon a piece of plate glass situated upon the plate of the compressorium.

The plate glass is usually fixed in the hole in the brass plate, but it is more convenient to have a ledge attached to one side, so that an ordinary plate glass slide may rest upon it. With such an arrangement, the tissue to be examined can be placed as may be thought desirable, upon any part of the glass before it is removed to the compressorium.

Several modifications of this instrument have been proposed and are well adapted for certain special purposes, but I think for ordinary use the form just referred to will be found quite as useful as a more expensive one.

A very convenient form is employed by M. Quatrefages, in which it is possible to examine the object upon either side.

The *cell* or *animalcule cage* also serves the purpose of a compressorium when a very great amount of pressure is not required. It is important that the shoulder upon which the cover fits should be at least as wide as this one, otherwise when the glasses are not cleaned immediately after use, solutions which have been examined are apt to dry and prevent the removal of the cover without much trouble.

Of the Importance of Examining the Same Objects in Various Ways.

Many objects require examination in several distinct ways before an accurate idea of their general structure can be obtained. It is in many instances of the utmost importance to examine a body by *reflected light* as well as by *transmitted light* and to observe the peculiarities of structure when it is surrounded with *air*, or immersed in *water*, or in a highly refracting fluid, such as *glycerine*, *oil*, *turpentine*, or *Canada balsam*. Not less valuable is the information we derive from the application of certain *chemical reagents*, to the consideration of which I shall devote some time in a future lecture. The microscopical observer must, however, feel that in order to make out the exact nature of any texture it is necessary to subject it to various different processes of observation, and to the action of certain chemical reagents, according to the *trans-*

parency or opacity, density, refractive power, and *chemical composition* of the specimen. So also he must submit the object to examination with *high powers* and *low powers.*

Appearance of the Same Object examined in Air, Water, and Canada Balsam, by Transmitted Light, and under the influence of Reflected Light and Polarized Light.—Under these five microscopes have been placed specimens of the same structure (spherical crystals of carbonate of lime) magnified in the same degree.

In Air.—In the first they are shown by *transmitted light* in air mounted in the dry way, and you will notice how very dark and thick their outline appears, and how impossible it is to make out the ultimate arrangement of the cyrstalline mass.

In Water.—In the second microscope the crystals are seen in water. The outline is still very dark and thick, but a few lines may be observed radiating from the centre of the crystals towards their circumference, although not very distinctly.

In Canada Balsam.—In the third microscope the crystals are shown immersed in Canada balsam. Here the outline appears as a sharp well-defined line. A vast number of narrow lines are seen radiating from the centre of the crystal towards its circumference, which shows that it is really made up of a congeries of minute acicular crystals.

By Reflected Light.—In the fourth microscope the crystals may be examined by reflected light. Their globular form, and yellowish colour, are very distinctly seen, and you may observe that the surfaces of the crystals generally are slightly rough, while some appear to be covered by minute elevations.

By Polarized Light.—Under the fifth microscope another preparation of the crystals is seen under the influence of polarized light. Each crystal exhibits a black cross which alters its position and appearance as the *analyzer* is rotated. These important points might be illustrated by a vast number of other substances, and I cannot advise you too strongly to subject various microscopical structures to examination in *air, water, and Canada balsam,* and by *direct* or *reflected,* as well as under the influence of *transmitted* light, and in some cases by *polarized light.*

PLATE XX.

Fig. 88.

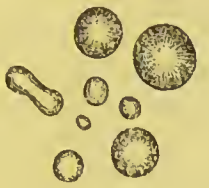


Fig. 89.

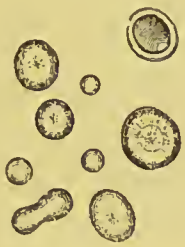


Fig. 90.



Fig. 91.

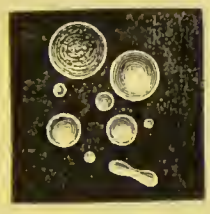


Fig. 92.

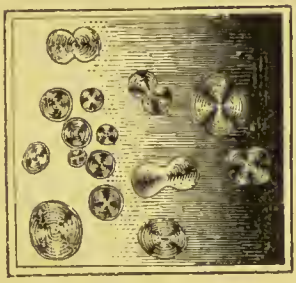


Fig. 93.

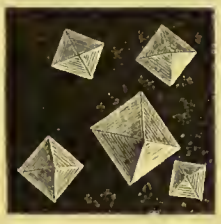


Fig. 94.

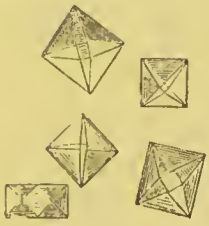


Fig. 95.

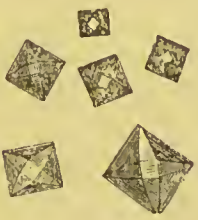


Fig. 96.

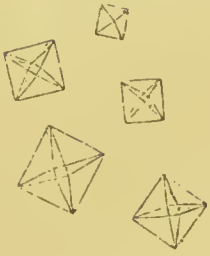


Fig. 97.

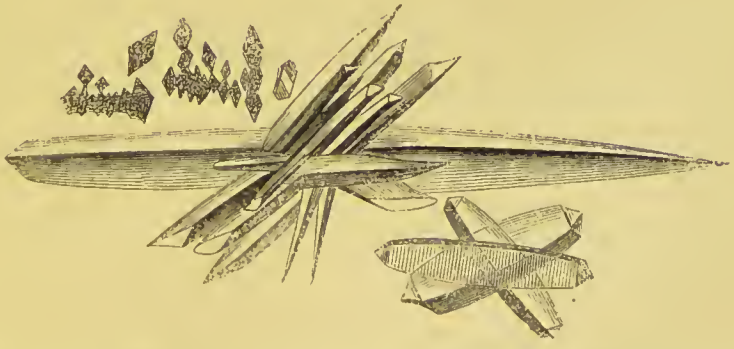


Fig. 88. Spherical crystallines of carbonate of lime, examined by transmitted light in air (p. 58).
Fig. 89. The same in water. Fig. 90. The same in Canada balsam. Fig. 91. The same under the influence of reflected light. Fig. 92. The same under the influence of polarized light (p. 58).
Fig. 93. Octohedral crystals as seen by reflected light Fig. 94. The same in air by transmitted light. Fig. 95. In water. Fig. 96. In Canada balsam. The thin lines in the last case are caused by the refractive power of the crystal and that of the medium in which it is immersed being nearly equal.
Fig. 97. To illustrate the appearance of crystals when examined in their own mother-liquor.

[To face page 58.]

LECTURE VI.

On the examination of tissues and their preservation as permanent objects.—General considerations with reference to the nature of the medium in which tissues should be placed for examination.—*Examination and preservation of structures in air.—Examination and preservation of substances in aqueous fluids.—Examination and preservation of soft tissues;* musele.—Arrangement for pressing down the thin glass eover upon the preparation while the Brunswick black is drying.—Examination of vegetable tissues.—Of the eireulation in the cells of valisneria.—*Examination and preservation of objects in Canada balsam.*—Examination of hard tissues; bone.—*Air-bubbles.—Oil-globules.—Of the separation of deposits from fluids.*—The pipette.—Removing the deposit with the pipette.—Examination of infusoria.—Vorticellæ.—Zoophytes.—On separating the coarse from the finer particles of a deposit.—Method of obtaining the silicious skeletons of lower organisms.—Wash-bottle —Of keeping preparations in the cabinet.

Of the Examination of Tissues and of their Preservation as Permanent Objects.

Gentlemen,—It is not easy to lay down rules with regard to the proper medium for mounting particular objects and for displaying their internal structure in the most satisfactory manner. There are, however, some general considerations which it may perhaps be desirable to bring under your notice.

General Considerations with Reference to the Nature of the Medium in which Tissues should be placed for Examination.—If the structure be dry and very thin, or if we require only to make out any general points with reference to its outline, or the character of its surface, it may be examined in air. So also many structures examined by low powers, and by reflected light, exhibit their general structure very satisfactorily when mounted perfectly dry.

If the structure be delicate and moist, and readily destroyed by careless manipulation, it should be examined in some aqueous fluid when quite fresh. The character of the fluid will vary in different cases. Water answers well in many

instances, but the microscopical characters of some textures are completely altered by water, or even altogether destroyed by it. Others are so dark and opaque that they are not well displayed in water. Cellular structures become distended by it. In consequence of containing a denser fluid in their interior, the more limpid water passes through the cell wall, and mixes with the cell contents. The cell thus becomes distended by this flowing in or endosmosis, and the process may go to such an extent as to cause the rupture of the cell and the escape of its contents. To prevent this result, it is necessary to immerse the structure in some fluid approaching to that in its interior in density. A little white sugar may be dissolved in the water with this end, or a little glycerine may be added to it for the same purpose. Perhaps of all substances soluble in water, glycerine is one of the most useful to the microscopist. With glycerine he may obtain a fluid of any density, and of various degrees of refracting power. Moderately strong solutions of glycerine preserve organized structures for any length of time. Glycerine is to moist tissues, what Canada balsam is to textures which are capable of being dried, without their structures being impaired. The most dense, opaque, and ill-defined structures, immersed in glycerine become clear and transparent ; and anatomical peculiarities which were before indistinct or not observable, become demonstrable without difficulty. Another advantage is, that by the addition of a little water all the original characters of the tissues are restored.

Canada balsam exerts a similar effect upon many textures in rendering them transparent, but it is of course only applicable to those structures which are not altered or destroyed by drying, and its use, therefore, is very limited. Glycerine does not possess these disadvantages, and in it any moist tissue may be placed. Glycerine might be termed the Canada balsam of moist tissues.

Examination and Preservation of Structures in Air.

Many structures naturally existing dry are often preserved in air, but the most delicate points in their anatomy can seldom be demonstrated without immersion in an *aqueous fluid*, or in *oil*, *turpentine*, or *Canada balsam*.

Any specimen examined, or preserved permanently, as a dry object in air must be protected from dust by being covered with thin glass, and the pressure of the latter upon the specimen must be prevented by the interposition of small pieces of cardboard at the edges of the thin glass, slightly thicker than the specimen itself. Objects may be mounted in the dry way in many of the cells described in my fourth lecture ; but a simple cell made of wood or cardboard is sufficient for all practical purposes. The round vulcanized India-rubber rings cemented to the glass slides make capital cells for mounting such preparations.

The thin glass cover must be attached by a little very thick gum, or by a paste made of gum and flour or chalk.

Among unorganized substances, there are many objects which may be mounted or preserved with advantage in air. Many crystalline substances found native, and crystals derived from the organic and inorganic kingdoms artificially prepared, may be examined or preserved permanently in air. Many of these present very beautiful appearances. Arsenious acid, common salt, benzoic acid, uric acid, crystals of the vegetable alkaloids, such as salicine and many crystalline salts, bone, teeth, hair, the scales of butterflies and other insects, are examples of structures which may be examined in air and mounted dry.

The general structure of many vegetable preparations may be shown in this simple manner. The petals of many flowers, different forms of vegetables, cellular and vascular tissue, the epidermis, hairs, and other parts of plants, the petals of flowers, the seeds and seed-vessels, spiral fibres, the stones of fruits, sections of wood, of the pith from the stem of various plants, pollen, the spores of ferns, mosses, and fungi, are examples of vegetable preparations which may be examined and preserved in air.

Examination and Preservation of Substances in Aqueous Fluids.

I have already drawn your attention to the most important points to be borne in mind, with reference to the examination of substances in aqueous fluids. In choosing a fluid in which the specimen is to be immersed, we must consider its chemical

composition, its transparency, and its refractive power. The different preservative solutions described in my third lecture (page 34) are applicable for the preservation of a variety of objects in fluid. If we wish for a fluid closely resembling water, but possessing the property of preserving the specimen, we may use the *solution of naphtha and creosote*, or *naphtha and water*. If we require a fluid of higher specific gravity, some of the saline solutions, diluted with a proper quantity of water may be used. If we wish for a solution which refracts highly, we may employ glycerine, or a mixture of glycerine and gelatine; while, if we require a fluid which has the property of hardening the structure, we may immerse it in a solution of *chromic acid*, *corrosive sublimate*, or *diluted alcohol*.

In all cases the substance should be immersed for some time in the fluid, in which it is to be preserved, before being mounted permanently. The cell made of *Brunswick black* or the thin glass cell, or other forms which I showed you in my third lecture, may be chosen according to the dimensions of the specimen. The object and fluid being placed in the cell, the thin glass cover is applied, with the precautions which I shall presently advert to, the superfluous fluid is removed with a piece of blotting paper, or a soft cloth, and after the edges have been allowed to dry a little, they are anointed with a thin layer of Brunswick black.

Almost every organized structure, and especially the soft moist tissues of the bodies of animals, may be advantageously preserved in fluid. The solution which is employed for preserving a structure should resemble as nearly as possible in density and refractive power, the fluid which bathed it during life.

Examination and Preservation of a Soft Tissue; Muscle.—Suppose a portion of this muscular fibre is to be examined under the microscope. I remove a small piece with a pair of very fine scissors, and place it carefully upon this glass slide. With the aid of two needles I tear it into very small shreds, and then moisten it with a little water dropped upon it from the finger, or from a pipette, or from the wash-bottle; or I add to it, instead of water, a drop of serum, of syrup, or of glycerine; next a square or circular piece of thin glass held in a pair of fine forceps is gently breathed upon and applied to

the surface of the liquid, being brought into contact with it, first on one side, and afterwards allowed to fall down very gradually with the aid of a needle or piece of fine wire placed underneath one edge, until it is completely wetted. Lastly, any superfluous fluid is to be absorbed by a cloth, or a small piece of fine sponge or blotting paper, and the slide placed in the field of the microscope for examination.

It is important to prevent the entrance of air-bubbles during the application of the thin glass cover; and if any are visible in the tissue or surrounding fluid before it is applied, it will be better to wait a few minutes until they rise to the surface of the liquid and burst. While time is allowed for this to take place the specimen should be covered with a small glass shade to prevent dust falling upon it.*

It is advisable not to remove too much of the fluid, for fear the thin glass should press so heavily upon the preparation, that the several structures of which it is composed would be squeezed together and the specimen rendered confused. You will find it very useful to place a piece of hair or hog's bristle, as I am now doing, between the thin glass and the glass slide, by which means too great pressure will effectually be prevented. The same effect is obtained by using a glass cell, but it will be found, I think, that it is more convenient to pursue the plan just described in the mere *examination* of most tissues under the microscope than to place them in a glass or other cell.

If the specimen is to be preserved permanently it should be immersed in the fluid in which it is intended to remain for several hours previous to being mounted, so that it may be thoroughly saturated with it in every part. The fluid may be placed in a moderately deep cell, in a watch-glass, or in a cup, in one of the palets used by artists, from which it may afterwards be removed to the slide. The thin glass having been applied, and all superfluous fluid removed, a thin layer of Brunswick black is to be carefully placed round the edge so as to cement the thin glass to the slide. When this is dry

* The small green forcing glasses, and the small green glass saucers, which can be purchased for a few pence at many of the glass shops in London, will be found most convenient for many microscopical purposes.

other layers are to be applied successively until the joint is considered quite tight. The cement adheres better to the glass-slide if it is roughened previously by grinding in this part, or it may be scratched with the writing diamond where the cement is to be placed. All objects, however, to be preserved permanently in fluid should be placed in a cell, because there is a much better prospect of their being kept permanently, than when placed upon the glass slide in the manner employed for examining the specimen temporarily. The chance of air getting into the cell is much diminished if the cement which is used possesses slight elastic power, so as to admit the necessary alteration, which necessarily takes place in the volume of the fluid, under variations of temperature. Mr. Brooke always adds a few drops of a solution of India-rubber to the Brunswick black and this admirably fulfils the end in view.

Arrangement for Pressing Down the Thin Glass Cover upon the Preparation while the Brunswick Black is Drying.—There are some substances which require slight pressure to display their peculiarities, and it is necessary to be provided with an arrangement for keeping down the glass cover until the cement which is to fix it in its place is dry. A very simple way of effecting this is, to place a small piece of wood, about an inch in height, upon the cover. This may be fixed in its place by passing a piece of thread over it, and tying it at the back of the slide ; or the wood may be kept in its place by a vulcanized India-rubber ring. My friend Mr. White has devised a very simple and ingenious apparatus for this purpose. It consists of a bent lever, which, by acting upon a screw, can be forced down upon the thin glass with the amount of pressure required. The slide must be allowed to remain in this apparatus until the varnish be thoroughly dry. The compressorium may also be employed for the same purpose, by inserting a small piece of cork between the thin glass to which the pressure is to be applied, and the glass of the compressorium itself.

Examination of Vegetable Tissues.—The examination of vegetable tissues is conducted upon the same general principles as that of animal textures. The spiral vessels of plants can in many instances be obtained by boiling the stem of the plant for some time in water. Those of rhubarb are very

Fig. 98. PLATE XXI.

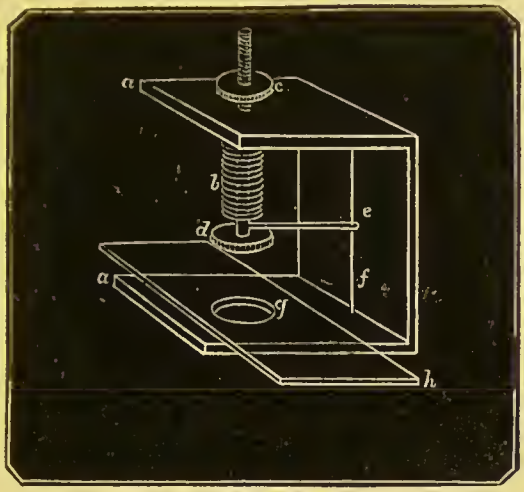


Fig. 99.

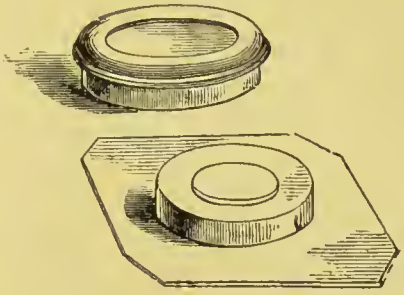


Fig. 100.

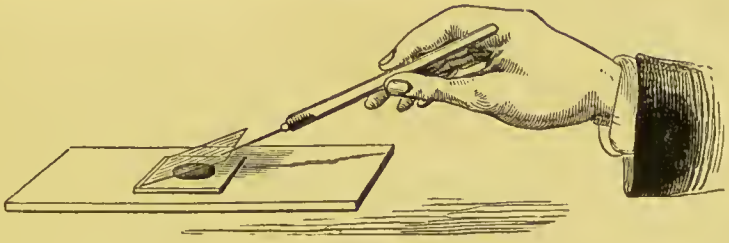


Fig. 101.

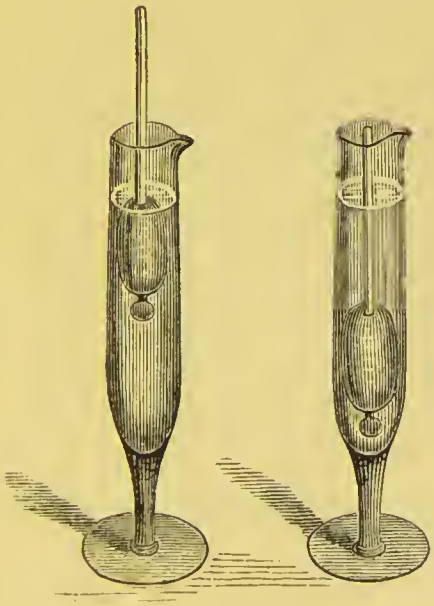


Fig. 102.

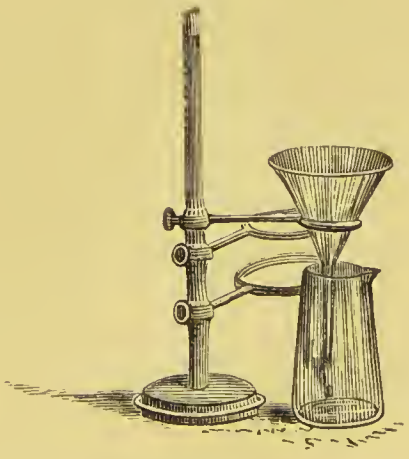


Fig. 98. Apparatus for pressing down the thin glass cover while cements are drying, arranged by the Rev. G. Isbell.
Fig. 99. Animalcule cage for examining strata of fluids of different degrees of thickness (pp. 46, 57).
Fig. 100. To illustrate the manner in which the thin glass cover is to be allowed to fall gradually upon an object to be mounted in fluid (p. 63).
Fig. 101. Glasses for taking the specific gravity of fluids and for allowing deposits to subside (p. 68).
Fig. 102. Shows how filtration may be conducted.

[To face page 64.]

large, and may be selected for examination. Many plants exhibit circulation in the cells of which they are composed, and are very favorite microscopic objects.

Examination of the Circulation in the Cells of Vallisneria.—Suppose we wish to examine the circulation in the cells of this thin leaf of the vallisneria spiralis, we may proceed as follows:—A small portion is cut off from the plant, and a very thin slice removed from the surface with a sharp thin knife, so that the cells within the leaf may be brought clearly into view. The manipulation to which the piece is thus necessarily subjected, has the effect of retarding or even of stopping the circulation for a time. If, however, the section be kept for a short time in water it soon recommences. It is a good plan when we wish to exhibit specimens of this beautiful plant, to cut several sections of the required size, and place them in a small bottle of water in a warm room or in the pocket, for an hour or more before they are submitted to microscopical examination.

Here is a portion of seaweed which I might wish to preserve permanently. It should be allowed to soak for some time in pure water. Small pieces may then be removed and transferred to glycerine, in which fluid they may be preserved permanently. The pieces should, however, always be permitted to soak for some time before they are finally mounted. Some of the most beautiful vegetable preparation which I have seen have been mounted in glycerine. The mixture of gelatine and glycerine will also be found a good medium for mounting many vegetable structures, and chloride of calcium forms a good preservative fluid in many instances. Creosote fluid, very dilute spirit and water, and even simple distilled water will preserve some vegetable tissues for a great length of time. The pith of the stem, the epidermis, and many other vegetable tissues may be preserved as dry objects very satisfactorily.

Examination and Preservation of Objects in Canada Balsam.

Canada Balsam has long been a favorite medium for the preservation of microscopical specimens, on account of its

penetrating and highly refracting powers. Turpentine possesses very similar properties, but from being a limpid fluid, it is far less useful than Canada balsam. All preparations to be mounted in Canada balsam must be thoroughly dried first. The desiccation must be effected by a temperature of not more than from 100 to 200 degrees. For the purpose of drying tissues, we may employ the water-bath alluded to in page 26, or we may place the specimen under a bell-jar close to a basin of strong sulphuric acid or chloride of calcium, which substances have the power of absorbing moisture in an eminent degree. Many textures in process of drying include a number of air-bubbles in their interstices, and it is often very difficult to remove these. To effect this object, the preparation may be allowed to soak some time in turpentine, and the removal of the air is often much facilitated by the application of a gentle heat. If the air cannot be removed in this manner, the preparation immersed in turpentine, may be placed under the receiver of an air-pump. As the pressure is removed the air rises to the surface and the fluid rushes in to supply its place.

When the specimen has been thoroughly dried, and the air removed, it may be slightly moistened with turpentine before it is placed in the balsam.

I will now endeavour to mount this thin section of bone in Canada balsam, so that you may see the successive steps of the process. The glass slide having been warmed upon the brass plate, a small quantity of Canada balsam is removed upon the end of a piece of iron wire. By gently warming it, it becomes perfectly fluid, and may be allowed to drop in its proper place upon the glass slide. Or the pot containing the Canada balsam may itself be warmed, and a drop of the fluid balsam placed upon the slide. I now take the preparation, and with the aid of a needle, place it in the drop of balsam, so that it may be thoroughly wetted by it in every part. Upon the surface of the balsam, a few air-bubbles may be observed, and by moving the slide from side to side, with a slight rotatory movement while the balsam is quite fluid, the bubbles may be seen to collect in one spot upon the surface. They may be made to burst by the application of a warm needle, or completely removed by touching them with a cold wire to

PLATE XXII.

Fig. 103.



Fig. 104.



Fig. 105.

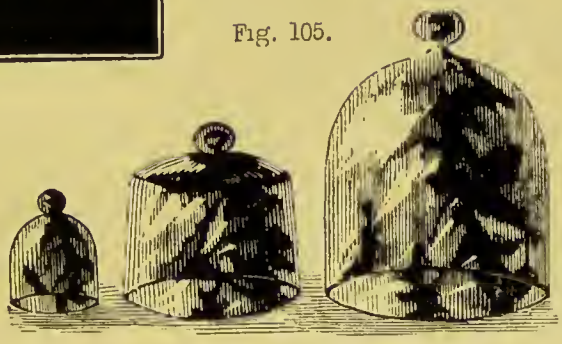


Fig. 106.

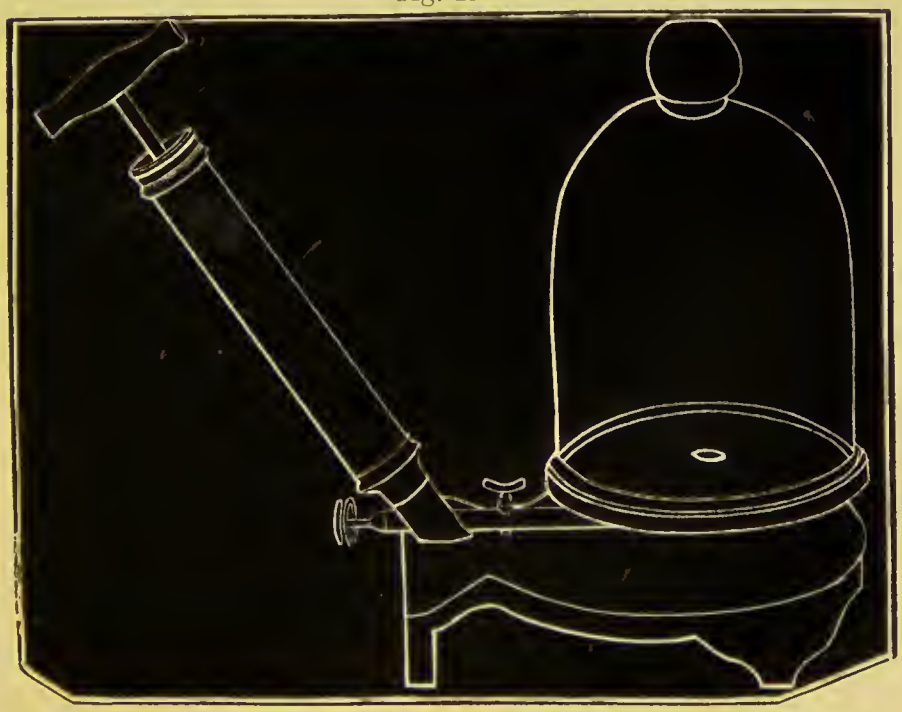


Fig. 103. Vessel for holding Canada balsam (p. 32).
Fig. 104. Vessel for holding gum, gold size, varnish, glycerine, &c. (p. 32).
Fig. 105. Glass shades for protecting specimens which are being mounted from dust (p. 63)
Fig. 106. Small air-pump for removing air from the interstices of a tissue (p. 66).

[To face page 66.]

which they will adhere. All bubbles having been removed, I take the thin glass, which has been perfectly cleaned and slightly warmed on the brass plate, in a pair of forceps,—and gently allowing one side of it to come in contact with the balsam, I permit it to fall very gradually upon the specimen, in such a manner that the balsam gradually wets the thin glass, without including air-bubbles. I then press it down slightly with a needle, and place the slide in a warm place. The superabundant balsam may be scraped away, and the preparation when cold, cleaned with a little turpentine, and a soft cloth, or piece of wash-leather.

These feet of the fly and other specimens might be mounted in the same manner.

The shells and hard parts of the covering of many of the lower animals, the palates of various mollusks such as the limpet, and many fresh-water species, the coriaceous coverings of insects, their antennæ, stings, eyes, feet, wings, and scales of their wings, the tracheæ penetrating every part of their organism with their spiracles or external openings, and in some cases the entire insects themselves, the scales of fishes, sections of bone, teeth, horn, hoofs, claws, nails, specimens of various kinds of hair, are examples of objects derived from the animal kingdom which may be mounted in this manner.

Examination of Hard Tissues.—Bone may be made to present very different characters in Canada balsam. Thus, if the specimen in this microscope be examined, you will observe in every part of the section, small black spots of irregular shape, from which a number of minute dark lines radiate, and inosculate pretty freely with corresponding lines from other spots.

In the other section no such appearance is observable, but the specimen appears clear, and its structure nearly uniform throughout. The first specimen is mounted in old viscid balsam ; the second was put up in fluid balsam, after having been previously wetted with turpentine.

The cause of these differences is interesting and worthy of attentive study. The little black spots (lacunæ) and dark lines (canaliculi) were originally considered to be small solid bodies, and the spots were improperly termed *bone corpuscles*. In truth, they consist of little cavities in the bony tissue,

containing air. In the second specimen the highly refracting oil of turpentine has passed up the canaliculi and entered the lacunæ, thus rendering them invisible. These cavities, in the fresh bone, contain fluid which nourishes the osseous tissue, but when the bone becomes dry, this fluid dries up, and air rushes in to the lacunæ and canaliculi to supply its place. The great difference between the refracting power of the air contained in these little cavities, and the osseous tissue in which they are contained, gives rise to their dark appearance.

Air Bubbles in water have a very wide dark outline: indeed, small air bubbles take the form of round black spots. This appearance is very characteristic, and every observer ought to be thoroughly familiar with it.

Oil Globules also present a peculiar and well-known appearance. The outline is sharp, and dark, and well defined, but not nearly so wide as that of the air bubble, because the difference of the refractive power between the oil and the fluid, although very great, is much less than that which exists between the air and the fluid medium which contains it. I must ask you to compare carefully the air bubbles and oil globules which are placed in these microscopes.

These points are of great interest and importance, and everyone engaged in microscopical enquiries should bear in mind the great influence which the medium in which a specimen is examined, exerts upon its appearance in the microscope.

On the Separation of Deposits from Fluids.

In order to ascertain the nature of a deposit suspended in a fluid, there are two or three important but very simple processes to be borne in mind. The first object is to separate the deposit as much as possible from the surrounding fluid, to collect it into a small space. Diffused as it often is through a large bulk of fluid, you would scarcely be surprised if you failed to find what you were looking for when you placed a drop of the fluid under the microscope.

Conical Glasses.—In order to collect the deposit for microscopical examination, the fluid is placed in a conical glass, the lower portion of which is narrow, but which at the same time does not terminate in a point but in a slightly-rounded extremity. After standing for some hours, the deposit falls

PLATE XXIII.

Fig. 107.

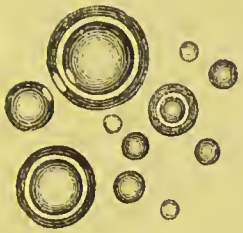


Fig. 108.

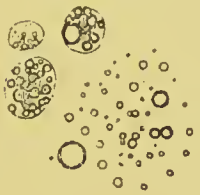


Fig. 109.

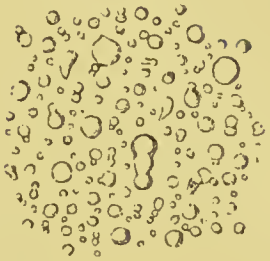


Fig. 110.

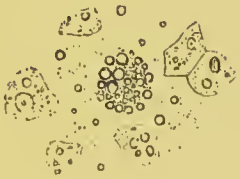


Fig. 111.



Fig. 112.

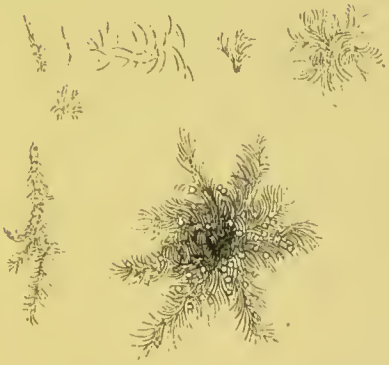


Fig. 113.



Fig. 114.

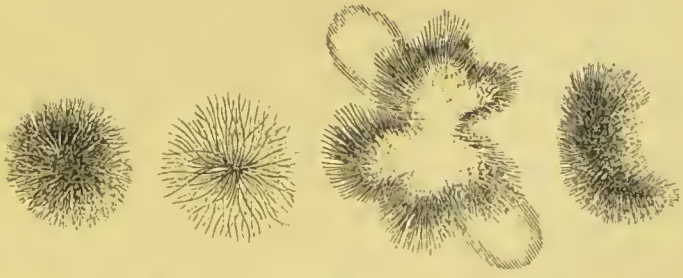


Fig. 107. Air bubbles of various sizes in water (p. 68).
Fig. 108. Oil globules, some free, others in envelopes (p. 68).
Fig. 109. Oil globules from milk. In some the investment of casein is dissolved and they are coalescing (p. 102).
Fig. 110. Liver cells containing oil-globules. In the centre is seen a collection of oil-globules not surrounded by any envelope (p. 101).
Fig. 111. Blood corpuscles.
Fig. 112. Crystals of margaric acid.
Fig. 113. Crystals of stearic acid.
Fig. 114. Crystals of margarine separating from the oily fat or elaine. [To face page 68.]

to the narrow portion of the glass, and may be removed with the pipette.

The Pipette consists of a glass tube, about ten inches in length, the upper extremity being slightly enlarged, so that the finger may be conveniently applied to it, and the lower orifice contracted, so as to be about one-tenth of an inch in diameter. It is convenient to have a ridge around the glass tube, about three inches from its upper extremity.

Removing the Deposit with the Pipette.—The removal of the deposit by the pipette is exceedingly simple. It is held by the middle finger and thumb, while the index finger is firmly applied to its upper extremity. The point is next plunged beneath the surface of the fluid and carried down to the deposit, a portion of which will rush up the tube if the pressure of the finger upon the upper extremity be slightly diminished. The deposit having entered the tube, the pressure is re-applied, and the deposit contained in the pipette can be removed from the fluid.

Where the deposit is exceedingly small in quantity, and diffused through a great bulk of fluid, a slight modification of the above plan must be resorted to. The pipette containing as much of the deposit as can be obtained, is removed from the glass vessel containing the fluid. Its contents are prevented from escaping by the application of the finger to its lower orifice. The upper extremity is then occluded with a small cork. Upon now removing the finger from the lower orifice, of course no fluid will escape. The pipette is allowed to stand with its mouth downwards upon the glass slide, in which position it may be permitted to remain some hours, either being suspended with a string or allowed to lean against some upright object. It is obvious that under these circumstances the most minute deposit contained in the fluid will gravitate to its lower part, and be received upon the slide, without the escape of much of the fluid.

In this manner any insoluble substances diffused through fluids can be easily collected for the purposes of examination. In collecting shells of the diatomaceæ for microscopical examination they are often diffused through a considerable quantity of water, allowed to subside, and obtained in the manner above described.

Examination of Infusoria, &c.—Suppose I wish to submit some of the animalcules in this water to microscopical examination, I should proceed as follows. A drop of the water must be removed with a pipette, or upon a glass rod, or with the finger, and placed upon the glass slide. A bristle or thin piece of paper is placed in such a position as to prevent the thin glass from coming into too close contact with the slide ; or the drop may be placed in a Brunswick black, or thin glass cell ; or the animalcule cage previously described (page 46) may be used with advantage. By the latter instrument the larger infusoria may be kept still in a particular position for the purposes of examination.

Vorticellæ and *Rotifers*, or wheel-animalcules, may often be obtained by placing a small piece of a plant which has been allowed to remain in the same water for some time, with a drop of the fluid, in a glass cell, observing the precautions before alluded to (page 63). These animalcules are often found attached to the edges of the plant in considerable number.

Zoophytes.—Fresh-water and marine Zoophytes, too large to be placed in the small cells, may be examined in flat watch glasses or in one of the larger cells alluded to in page 44.

These may be examined with low powers (two inch, one inch) without any thin glass cover, but where the higher powers are employed a piece of thin glass must be applied in such a manner as to cover that part of the vessel in which the animals are situated, without preventing a certain proportion of the fluid from being exposed to the air ; for if exposure to the air were prevented, the animals would soon exhaust all that dissolved in the small quantity of water in which they were imprisoned, and die of suffocation.

The troughs described in page 46 are also of great use for examining Zoophytes.

On Separating the Coarse from the Finer Particles of a Deposit.—Many deposits, by being diffused through a large quantity of water, may be divided into several portions. The fluid, with substances suspended in it, is well stirred, and, after being allowed to stand for a very short time, all but the deposit is poured off into another vessel. In this the fluid is again allowed to stand for a short time, and again poured off. This process may be repeated several times. In the first glass, only

PLATE XXIV.

Fig. 115.



Fig. 116.

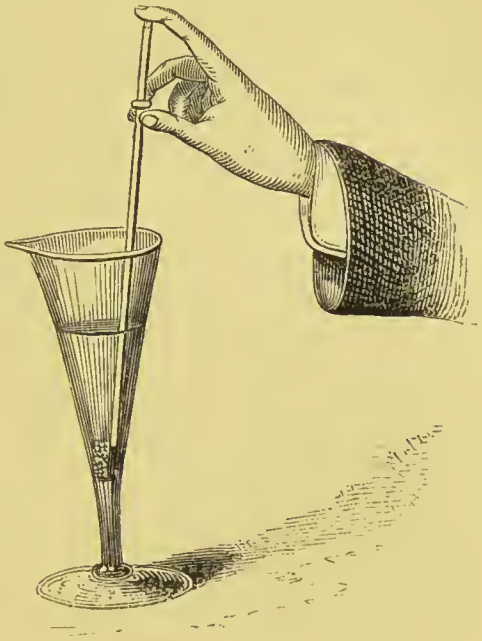


Fig. 117.

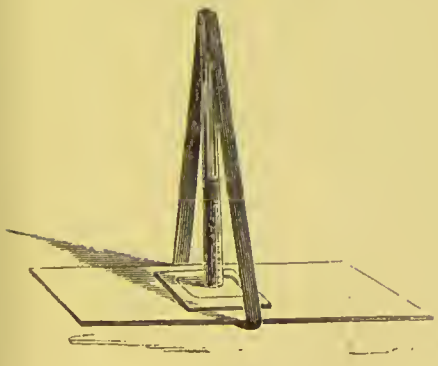


Fig. 118.

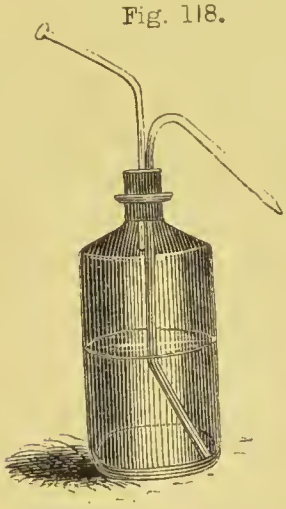


Fig. 119.

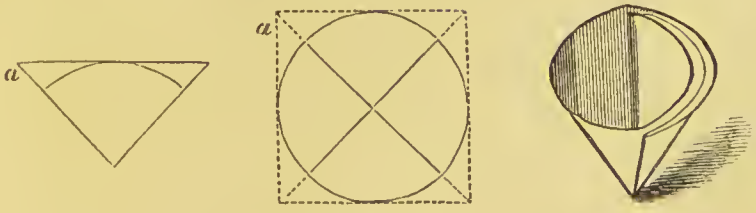


Fig. 115. Pipettes made of glass tube for separating deposits from fluids (p. 69).
Fig. 116. Illustrates the manner of using the pipette (p. 69).
Fig. 117. Shows the manner in which a very small quantity of deposit may be obtained from a fluid, by placing it in a test tube, and inverting it over the glass slide. It is kept in position by an India-rubber band shown in the drawing.
Fig. 118. Wash bottle (p. 71).
Fig. 119. Illustrates the manner in which filtering paper is to be folded for filtering.

[To face page 70.]

the coarser particles will be found ; in the second, slightly finer particles ; in the third, still finer ones, and so on ; a longer period being allowed for the subsidence in each successive case.

The coarse particles may also often be separated from finer ones by straining the deposit through muslin. Various preservative solutions, which I have already described, are applicable for preserving deposits from fluids. Many, again, may be mounted in Canada balsam.

Method of obtaining the Siliceous Skeletons of Lower Organisms.—The siliceous remains of the diatomaceæ may be separated from guano and other deposits as follows. The organic matter and carbonate and phosphate may be removed by boiling in nitric acid, and the remaining deposit diffused through water and collected as before described, but I much prefer to destroy the organic matter by burning the deposit in a platinum basin, and allowing it to remain for some hours at a red heat until the black carbonaceous matter has burnt off, leaving a pure white ash. The phosphates and carbonates may be removed with dilute nitric acid, and the deposit washed. In this way the shells are not liable to be broken as they are when the deposit is boiled for some time in strong acid.

Wash-Bottle.—In many operations the wash-bottle used by chemists is of great use, as by it a stream of water of any required degree of force can be easily directed to any particular point, either for the purpose of washing away foreign particles, or for removing part of the deposit itself. The wash-bottle is also of great use in preparing sections of soft tissues for observation (page 54). It is made by inserting a cork into an ordinary half-pint bottle. Through the cork pass two tubes, bent at the proper angle. The longest terminates in a capillary orifice, while its other extremity reaches down to the bottom of the bottle. The shorter tube reaches only to the lower part of the cork. By blowing through the shorter tube, air is made to press upon the surface of the water, which is thus driven up the longer tube and out at its capillary orifice.

The observer will also require a stock of *small tubes*, about two inches in length and a quarter of an inch in diameter, and several small *watch glasses*, of different sizes.

Of Keeping Preparations in the Cabinet.—Preparations mounted in the dry way, or in Canada balsam, may be kept upright, arranged in grooves, but all preparations mounted in fluid must be allowed to lie perfectly flat, otherwise there will be great danger of leakage. Cabinets holding several hundred specimens arranged in this manner may now be purchased, but if the observer is only provided with deep drawers, they may be made available for the purpose, by having a number of shallow trays made to fit them accurately. Each preparation hould be named as soon as it is put up, and it is convenient to keep a number of small gummed labels always at hand for this purpose. Once or twice in the year a new layer of Brunswick black should be applied, and the specimens carefully examined to see that no leakage has occurred.

LECTURE VII.

Of injecting.—Natural and artificial injections.—Transparent and opaque injections.—Instruments required for making injections.—Syringe, pipes, corks, bull's-nose forceps, needle for passing the thread round the vessel.—*Of opaque injections.*—Injecting cans.—Size.—Coloring matters.—Vermilion.—Chromate of lead.—White lead.—*Of transparent injections.*—Injecting with plain size.—Coloring matters.—Carmine.—Prussian blue.—Advantages of employing Prussian blue.—Composition of the Prussian blue fluid for making transparent injections.—Mercurial injections.—*Injecting the lower animals.*—Mollusca.—Insects.—Of the practical operation of injection.—Of injecting the ducts and secreting follicles of glands.—Of preparing portions of injected preparations for microscopical examination.

The arrangement of the minute vessels or capillaries distributed to various textures is not to be demonstrated in all instances by the usual methods of investigation, in consequence of the transparency of the walls of the tubes. Indeed, in

PLATE XXV.

Fig. 120.



Fig. 121.



Fig. 122.

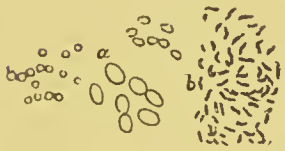


Fig 123.

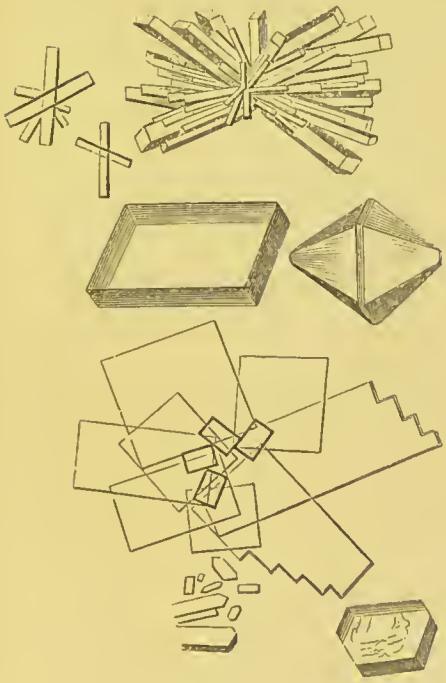


Fig. 124.



Examination of the lower animals for vegetable crystals.

Fig. 120. Sugar fungus in various stages of growth, after Dr. Hassall.
Fig. 121. Fungi germinating. × 215.
Fig. 122. Fungi and vibriones, or minute algæ (?)
Fig. 123. To illustrate the appearance of crystals under the microscope.
Fig. 124. Entozoa from the follicles of the skin. × 215.

[To face page 72.]

an ordinary examination of a tissue in the microscope, one often fails to detect the least trace of any structure which would be regarded as consisting of distinct tubes or vessels. Some authorities even yet maintain the opinion, that the capillaries are to be looked upon in the light of mere channels in the interstices of the tissues, rather than of tubular vessels with their own proper walls. If this opinion were correct we should hardly expect to meet with the perfectly circular outline which the section of an injected capillary vessel invariably presents.

Sometimes the capillary vessels remain turgid with blood after the death of the animal, and a *natural injection* results. There is a specimen of a good natural injection of the vessels of the stomach of the mouse, under one of the microscopes. Natural injections, however, are accidental and cannot be obtained in the case of every texture. In order, therefore, to investigate the arrangement of the vessels, it is necessary to resort to the process of *injection*, which consists simply of forcing into a vessel of convenient size, a certain quantity of colored material, which, after passing along the large trunk, shall penetrate into the smallest vessels and even return by the veins. The colouring matter employed may be *opaque* or *transparent*. In the first case the injected preparation can only be examined by *reflected light* as an *opaque object*, while transparent injections may be subjected to examination either by the aid of *transmitted light* or by *reflected light*. On the table are several examples of opaque and transparent injections in which different substances have been employed as coloring matters.

Instruments Required for Making Injections.—The different instruments required for making artificial injections are the following :—An *injecting syringe*, of about the capacity of one ounce or even half an ounce. The piston of the injecting syringe should be covered with two pieces of leather, which may be very easily removed and replaced. The first is applied and screwed down with a brass button. The piston is then passed down the tube and forced out at the lower opening. The second piece of leather is then put on, and fixed in its place with another button. In this syringe, made for me by Mr. Matthews, the piston consists entirely of metal. It

works well, and the necessity for re-leathering is obviated, but it is rather expensive.—*Pipes*, of different sizes, to insert into the vessels. The tube of the smaller pipes should be made of silver.—*Corks*, of the form which I now show you, for the purpose of plugging the pipes while the syringe is being filled with injecting fluid.—*Forceps*, of this form, which are known to surgical instrument makers as *bull's-nose forceps*, for stopping up any vessels through which the injection may escape accidentally.—*A Needle*, of the form of the *aneurism needle* used by surgeons, for passing the thread round the vessel to tie it to the pipe which is inserted into it. This needle may be made of an ordinary darning needle which has been carefully bent round after having been heated in the flame of a lamp. The *thread* which is used should be strong but not too thin, as there would be danger of its cutting through the coats of the vessel.

Of Opaque Injections.

Injecting Cans.—Size or gelatine is used as the material in which the colouring matter is suspended. It must be melted in a water bath and strained immediately before use. The copper injecting can forms a very convenient arrangement for melting the gelatine. There are five cans in this bath, so that injection may be very conveniently transferred from one into the other, while all may be kept warm over an ordinary lamp.

The Size should be of such a strength as to form a tolerably firm jelly on cooling. If gelatine is employed it must be soaked for some hours in cold water before it is warmed. About an ounce of gelatine to a pint of water will be sufficiently strong, but in very hot weather it is necessary to add a little more gelatine. It must be soaked in part of the cold water until it swells up and becomes soft, when the rest of the water, made hot, is to be added. Good gelatine for injecting purposes may be obtained for two shillings a pound.

Coloring Matters.—The most usual coloring matters employed for making opaque injections are the following—*Vermilion*, *Chromate of Lead*, and *White Lead*. Of these, vermilion affords the most beautiful preparations, but chromate of lead properly prepared is much cheaper, and it may

PLATE XXVI.

Fig. 125.



Fig. 126.

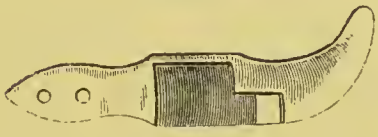


Fig. 127.



Fig. 128.

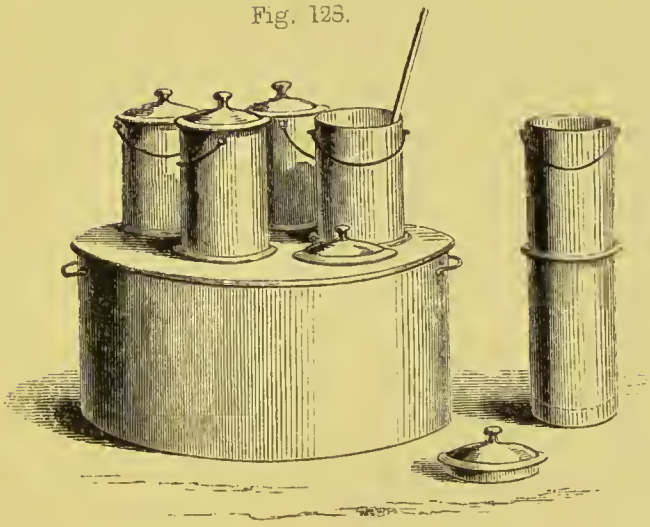


Fig. 129.

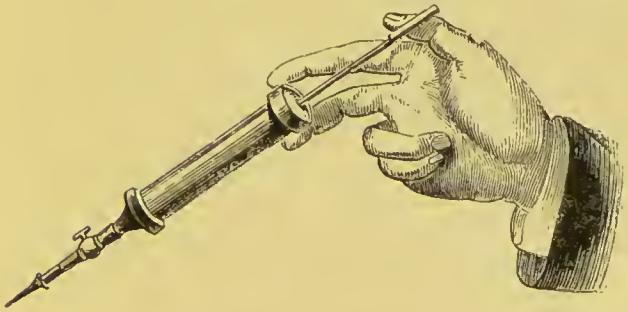


Fig. 130.

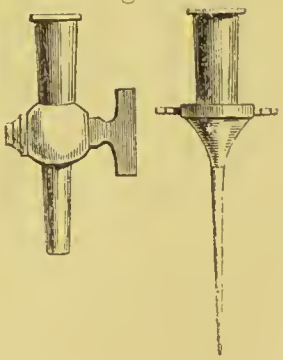


Fig. 131.

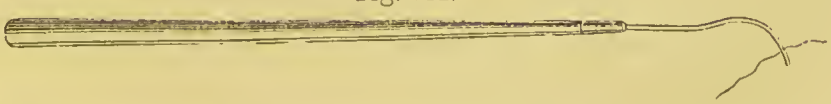


Fig. 125. Corks for stopping the pipe when the syringe is being refilled (p. 74).
Fig. 126. Bull's-nose forceps for closing an open vessel to prevent the escape of the injection (p. 74)
Fig. 127. Shows the manner in which the piston of the syringe is made.
Fig. 128. Injecting can for heating size. It may also be used as a water bath for drying objects, or for conducting evaporation (p. 74).
Fig. 129. Performing the operation of injecting (p. 80).
Fig. 130. Stopcock and injecting pipe, which fit on to the syringe (p. 74).
Fig. 131. Needle for passing thread round a vessel the cut end of which is to be tied on an injecting pipe (p. 74).

[To face page 74.]

be obtained in a state of more minute division. White lead forms a good coloring matter, but its density, and its tendency to become brown when exposed to the action of sulphuretted hydrogen formed in the decomposition of the tissues, are objections to its use.

Vermilion of sufficiently good quality can be purchased of all artists' colormen for six or eight shillings a pound. If upon microscopical examination a number of very coarse particles be found in the vermilion, it will be necessary to separate these by washing in water in the manner described when we last met (page 70).

The Chromate of Lead is prepared by mixing cold saturated solutions of acetate of lead and bichromate of potash. The yellow precipitate is allowed to settle, and after pouring off the clear solution of acetate of potash resulting from the decomposition, it is shaken up with water, again allowed to settle and mixed with strong size or gelatine. After being strained through muslin the mixture may be injected into the vessels.

The *Carbonate of Lead* or *White Lead* is prepared by mixing saturated solutions of acetate of lead and carbonate of soda. The precipitate is to be treated as the former one and mixed with size.

There should always be plenty of coloring matter in the size, otherwise the vessels do not appear to be uniformly filled, and it is better to employ a small syringe rather than a large one, as there is not so much chance of the colouring matter separating from the size before the mixture is forced into the vessels.

In all cases the coloring matter is to be well mixed with the size, and the mixture strained through muslin immediately before use.

The size of the ultimate particles of the different substances employed in making opaque injections is represented in this diagram, and if the different figures be compared with each other, it will be observed that those coloring matters which have been recently prepared are in a much more minute state of division than those which have been kept for some time. The appearances represented were obtained by examination with a power of 215 diameters.

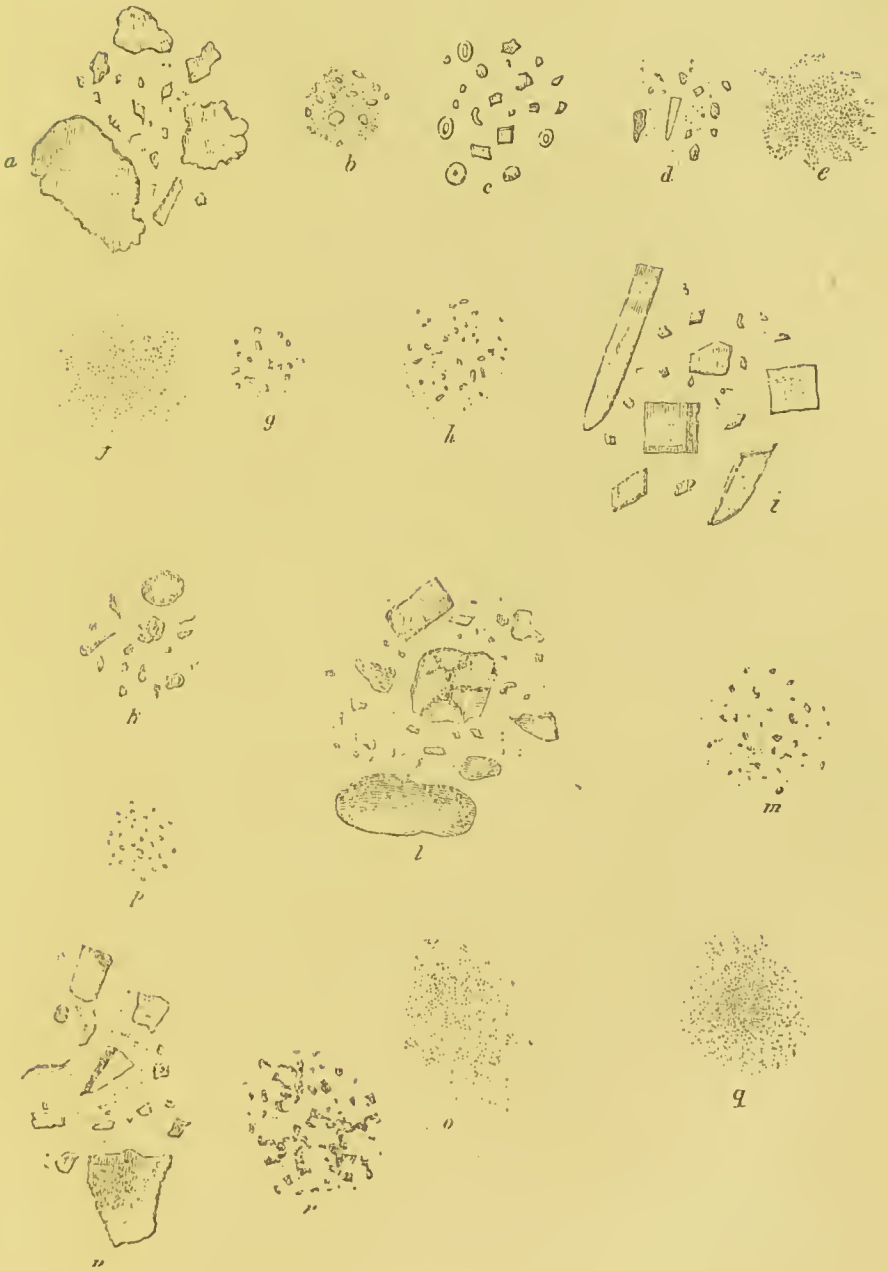
Of Transparent Injections.

Much more strongly, however, can I recommend to you the use of *transparent* fluids for making injections. It is true, that these are not likely to be so much admired by general observers as opaque injections. Indeed, it is not easy to find any object which will rival in beauty many tissues which have been freely injected with vermilion or chromate of lead ; although it must be confessed that from such preparations we learn but little save the general arrangement of the capillary vessels of the part, their capacity, and the magnitude of the meshes of the network. Of the relations which these vessels bear to the elementary structures which give to the texture under examination its peculiar properties, such preparations tell us nothing. Indeed the very mode in which they are prepared often precludes the possibility of seeing anything but the vessels themselves. In examining such specimens we cannot but admire the beautiful arrangement of the vessels, but at the same time we are not led to consider the ends which the arrangement serves, or the nature of the processes to which it is subservient, as the essential structures of which the tissues are composed are rendered quite invisible by this mode of injection. Opaque injections are for the most part only adapted for examination with low powers, while the tissues to which they are distributed can only be seen with the help of very high magnifying powers.

Comparatively little has been learnt from such preparations since the days of Leeuwenhoek. The injections in modern microscopical cabinets are undoubtedly more perfect than those of the older observers. The vessels are perfectly filled, but we learn little more from well filled than from partially filled vessels ; and although the former may be regarded as triumphs of mechanical skill and ingenuity, and deserve great praise, they teach us no more than a comparatively bad injection.

Transparent injections, on the other hand, though they fail to excite the wonder of the uninitiated, show us not only the general arrangement of the capillary network, but the precise relation which each little tube bears to the tissue with which it is in contact ; and we are necessarily led to think upon the

PLATE XXVII.



Colouring matter used for injecting, showing the comparative size of the particles. *a.* Precipitated chalk purchased in a dry state. *b.* Chalk recently precipitated. *c.* Whitening. *d.* Prussian blue, as purchased. *e.* Recently precipitated Prussian blue. *f.* Freshly precipitated carbonate of lead. *g.* Dried carbonate of lead. *h.* Freshly precipitated biniodide of mercury. *i.* Dried biniodide of mercury. *k.* Indigo. *l.* Vermilion, as purchased. *m.* Levigated vermilion. *p.* Pure carmine. *n.* Dried chromate of lead. *r.* Freshly precipitated chromate of lead (hot solutions of bichromate of potash and acetate of lead). *o.* Freshly precipitated chromate of lead (cold solutions). *q.* Lamp black. × 215 (p. 75).

[To face page 76.]

nature of those wonderful and highly complex changes which must be momentarily taking place between the tissue in which incessant decay and renovation are going on, and the blood which alike brings to it the pabulum for its nutrition and carries away the substances resulting from its decay, which are only separated from it by the excessively thin vascular wall through which this perpetual flowing in and out, and this constant solution and solidification, take place.

In order to make injected preparations for examination by transmitted light several different substances may be used as injecting fluids.

Injection with Plain Size.—A tissue which has been injected with plain size, when cold is of a good consistence for obtaining thin sections, and many important points may be learned from a specimen prepared in this manner which would not be detected by other modes of preparation. A mixture of equal parts of gelatine and glycerine is, however, much to be preferred for this purpose, and the specimen thus prepared is sure to keep well.

Coloring Matters for Transparent Injections.—The chief coloring matters used for making transparent injections are *carmine* and *Prussian blue*. The former is prepared by adding a little solution of ammonia (liquor ammonia) to the carmine, and diluting the mixture until the proper color is obtained, or it may be diluted with size.

The Prussian Blue consists of an insoluble precipitate, so minutely divided, that it appears like a solution to the eye. The particles of freshly prepared Prussian blue are very much smaller than those of any of the coloring matters employed for making opaque injections.

Advantages of Employing Prussian Blue.—I have lately been employing Prussian blue very much, and according to my experience it possesses advantages over every other coloring matter. It is inexpensive,—may be injected cold,—the preparation does not require to be warmed,—no size is required,—it penetrates the capillaries without the necessity of applying much force,—it does not run out when a section is made for examination,—neither do any particles which may escape from the larger vessels divided in making the section, adhere to it and thus render the section obscure,—a structure may

be well injected with it in the course of a few minutes. Specimens prepared in this manner may be preserved in any of the ordinary preservative solutions, or may be dried and mounted in Canada balsam, (but I give the preference to glycerine, or glycerine jelly,) and they may be examined with the highest magnifying powers. After having tried very many methods of making this preparation I have found the following one to succeed best.

Composition of the Prussian Blue Fluid for Making Transparent Injections.—

Glycerine	1 oz.
Wood, naphtha, or pyroacetic spirit	1½ drachms.
Spirits of wine	1 oz.
Ferrocyanide of potassium	12 grs.
Tincture of sesquichloride of iron	1 drachm.
Water	4 ozs.

The ferrocyanide of potassium is to be dissolved in one ounce of the water, and the tincture of sesquichloride of iron added to another ounce. These solutions should be mixed together very gradually, and well shaken in a bottle. *The iron being added to the solution of the ferrocyanide of potassium.* When thoroughly mixed, these solutions should produce a dark blue mixture, in which no precipitate or flocculi are observable. Next, the naphtha is to be mixed with the spirit, and the glycerine and the remaining two ounces of the water added. This colorless fluid is, lastly, to be slowly mixed with the Prussian blue, the whole being well shaken in a large bottle during the admixture. The tincture of sesquichloride of iron is recommended because it can always be obtained of uniform strength. It is generally called the *muriated tincture of iron*, and may always be purchased of druggists.

But while I recommend you to employ this fluid I wish you by no means to think that I consider it in all respects the best which may be obtained, indeed I hope that before long several colored fluids, equal or superior to this will be discovered.

Permit me, then, most earnestly to recommend all who are fond of injecting to employ transparent injections, and to endeavour by trying various transparent coloring matters, to discover several which may be employed for the purpose, for I feel sure that by the use of carefully prepared transparent

injections, many new points in the anatomy of tissues will be made out.

Of Injecting Different Systems of Vessels with Different Colours.—It is often desirable to inject different systems of vessels distributed to a part with different colours, in order to ascertain the arrangement of each set of vessels and their relation to each other. A portion of the gall-bladder in which the veins have been injected with white lead, and the arteries with vermilion forms a beautiful preparation. Each artery, even to its smallest branches, is seen to be accompanied by two small veins, one lying on either side of it.

In this injection of the liver, which, however, is not very perfect, four sets of tubes have been injected as follows :— The artery with vermilion, the portal vein with white lead, the duct with Prussian blue, and the hepatic vein with lake. There are many opaque coloring matters which may be employed for double injections, but I am acquainted with very few transparent ones, the employment of which affords very satisfactory results.

Mercurial Injections are not much used for microscopical purposes although mercury was much employed formerly for injecting lymphatic vessels and the ducts of glandular organs. The pressure of the column of mercury supersedes the necessity of any other kind of force for driving it into the vessels. The mercurial injecting apparatus consists of a glass tube, about half an inch in diameter and twelve inches in length, to one end of which has been fitted a steel screw to which a steel injecting pipe may be attached. The pipes and stopcocks must be made of steel, for otherwise they would be destroyed by the action of the mercury.

Injecting the Lower Animals.—The vessels of fishes are exceedingly tender, and require great caution in filling them. It is often difficult or quite impossible to tie the pipe in the vessel of a fish, and it will generally be found a much easier process to cut off the tail of the fish, and put the pipe into the divided vessel which lies immediately beneath the spinal column. In this simple manner beautiful injections of fish may be made.

Mollusca.—(Slug, snail, oyster, &c.) The tenuity of the vessels of the mollusca often renders it impossible to tie the

pipe in the usual manner. The capillaries are, however, usually very large, so that the injection runs very readily. In different parts of the bodies of these animals are numerous lacunæ or spaces, which communicate directly with the vessels. Now, if an opening be made through the integument of the muscular foot of the animal, a pipe may be inserted, and thus the vessels may be injected from these lacunæ with comparative facility.

Insects.—Injections of insects may be made by forcing the injection into the general abdominal cavity, whence it passes into the dorsal vessel and is afterwards distributed to the system. The superfluous injection is then washed away, and such parts of the body as may be required, removed for examination.

Of the Practical Operation of Injecting.—I propose now to inject a frog and the eye of an ox, in order that you may see the several steps of the process. We must bear in mind that a successful injection cannot be made until the muscular rigidity which comes on shortly after death, and which affects the muscular fibres of the arteries as well as those of the muscles themselves, has passed off. In some few instances in which the fluid does not necessarily pass through arterial trunks before it reaches the capillaries (as in the liver), the injection may be effected satisfactorily immediately after the death of the animal.

I shall inject these specimens with the Prussian blue injecting fluid, because it requires much less time for its performance than the ordinary method. The steps of the process are very similar in making the opaque injections, except that when size is employed, the specimen must be placed in warm water until warm through, otherwise the size will solidify in the smaller vessels and the further flow of the injecting fluid will be prevented. Soaking for many hours is sometimes necessary for warming a large preparation thoroughly, and it is desirable to change the water frequently. The size must also be kept warm, strained immediately before use, and well stirred up each time the syringe is filled.

In the first place the following instruments must be conveniently arranged :—

The syringe thoroughly clean and in working order, with pipes, stopcock and corks.

One or two scalpels.

Two or three pair of sharp scissars.

Dissecting forceps.

Bull's-nose forceps.

Curved needle threaded with silk or thread, the thickness of the latter depending upon the size of the vessel to be tied.

Wash-bottle.

Injecting fluid in a small vessel.

I will commence with the frog. An incision is made through the skin, and the sternum divided in the middle line with a pair of strong scissars; the two sides may easily be separated, and the heart is exposed. Next the sac in which the heart is contained (pericardium) is opened with scissars and the fleshy part of the heart seized with the forceps; a small opening is made near its lower part, and a considerable quantity of blood escapes from the wound—this is washed away carefully by the wash-bottle. Into the opening—the tip of the heart being still held firmly by the forceps, a pipe is inserted and directed upwards towards the base of the heart to the point where the artery is seen to be connected with the muscular substance. Before I insert the pipe, however, I draw up a little of the injecting fluid so as to fill it, for if this were not done, when I began to inject, the air contained in the pipe would necessarily be forced into the vessels, and the injection would fail.

The point of the pipe can with very little trouble be made to enter the artery. The needle with the thread is next carried round the vessel and the thread seized with forceps, the needle unthreaded and withdrawn, or one end of the thread may be held firmly, while the needle is withdrawn over it in the opposite direction. The thread is now tied over the vessel, so as to include the tip of the pipe only, for if the pipe be tied too far up, there is greater danger of its point passing through the delicate coats of the vessel.

The nozzle of the syringe, which has been well washed in warm water, is now plunged beneath the surface of the fluid, the piston moved up and down two or three times, so as to force out the air completely, and the syringe filled with fluid. It is then connected with the pipe, which is firmly held by the finger and thumb of the left hand, with a screwing

movement, a little of the injection being first forced into the wide part of the pipe so as to prevent the possibility of any air being included.

The pipe and syringe being still held with the left hand, the piston is slowly and gently forced down with a slightly screwing movement with the right, care being taken not to distend the vessel so as to endanger rupture of its coats. The handle of the syringe is to be kept uppermost, and the syringe should never be completely emptied, in case of a little air remaining, which would thus be forced into the vessels. The injection is now observed running into the smaller vessels in different parts of the organism.

I will now proceed to inject the ox-eye in the same manner. The pipe is inserted into this branch of artery close to the nerve. Two minutes will probably be sufficient to ensure a complete injection. In making an injection of the eye, if the globe becomes very much distended by the entrance of the injecting fluid, an opening may be made in the cornea to allow the escape of the aqueous fluid which will leave room for the entrance of the injection, and permit the complete distension of the vessels.

We will now examine these injections. A portion of the intestine of the frog may be removed with scissars, opened, and the mucous surface washed with the aid of the wash-bottle. It may be allowed to soak in glycerine for a short time, and then examined.

This portion of the delicate choroidal membrane which has been carefully removed in the same manner shows the vessels perfectly injected and in this preparation of the ciliary processes you will not fail to observe that all the capillary vessels are fully distended with fluid, although the injection was made so quickly.

Of Injecting the Ducts of Glands.—The modes of injecting which we have just considered, although applicable to the injection of vessels, are not adapted for injecting the ducts and glandular structure of glands ; for as these ducts usually contain a certain quantity of the secretion, and are always lined with epithelium, it follows that when we attempt to force fluid into the duct, the epithelium and secretion must be driven towards the secreting structure of the gland, which

is thus effectually plugged up with a colorless material, and there is no possibility of making out the arrangement of the parts. In such a case it is obviously useless to introduce an injecting fluid, for the greatest force which could be employed would be insufficient to drive the contents through the basement membrane, and the only possible result of the attempt would be rupture of the thin walls of the secreting structure and extravasation of the contents. As I have before mentioned, partial success has been obtained by employing mercury, but the preparations thus made are not adapted for microscopical observation.

I have long felt very anxious to inject the ducts of the liver in order to ascertain the manner in which they commenced in the lobule, and the precise relation which they bore to the liver cells. This has long been a point of dispute among microscopical observers, and many different and incompatible conclusions have been arrived at by different observers. In order to prove the point satisfactorily it was obviously necessary to inject the ducts to their minute extremities, which no one, as far as I was able to ascertain, had succeeded in doing satisfactorily. After death the minute ducts of the liver always contain a little bile. No force which can be employed is sufficient to force this bile through the basement membrane, for it will not permeate it in this direction. When any attempt is made to inject the ducts, the epithelium and mucus, in their interior, and the bile form an insurmountable barrier to the onward course of the injection. Hence it was obviously necessary to remove the bile from the ducts before one could hope to make a successful injection. It occurred to me, that any accumulation of fluid in the smallest branches of the portal vein or in the capillaries, must necessarily compress the ducts and the secreting structure of the liver which fill up the intervals between them. The result of such a pressure would be to drive the bile towards the large ducts and to promote its escape. Tepid water was, therefore, injected into the portal vein. The liver became greatly distended, and bile with much ductal epithelium flowed by drops from the divided extremity of the duct. The bile soon became thinner owing to its dilution with water which permeated the intervening membrane, and entered the

ducts. These long, narrow, highly tortuous channels were thus effectually washed out from the point where they commenced as tubes not more than 1-3000th of an inch in diameter, to their termination in the common duct, and much of the thick layer of epithelium lining their interior was washed out at the same time. The water was removed by placing the liver in cloths with sponges under pressure for twenty-four hours or longer. All the vessels and the duct were then perfectly empty and in a very favourable state for receiving injection. The duct was first injected with a coloured material. Freshly precipitated chromate of lead, white lead, vermilion, or other colouring matter may be used, but for many reasons to which I have alluded, the Prussian blue injection is the one best adapted for this purpose. It is the only material which furnishes good results when the injected preparations are required to be submitted to high magnifying powers. Preparations injected in this manner should be examined as transparent objects.* They may be mounted in the ordinary preservative fluids or in Canada balsam, but glycerine forms the most satisfactory medium for their preservation. A specimen of the ducts injected in this manner has been placed under the microscope, and specimens of the portal vein, of the ciliary processes, of the choroid, of the kidney, and some other tissues injected with the Prussian blue mixture, have been arranged for examination.

Of Preparing Portions of Injected Preparations for Microscopical Examination.—When thin tissues, such as the mucous membrane of the intestines or other parts, have been injected, it is necessary to lay them perfectly flat, and wash the mucus and epithelium from the free surface, either by forcing a current of water from the wash-bottle, or by placing them in water and brushing the surface gently with a camel's hair brush. Pieces of a convenient size may then be removed and mounted in solution of naphtha and creosote, in dilute alcohol, in glycerine, or in gelatine and glycerine. The most important points in any such injections are shown if the preparation be dried and mounted in Canada balsam. The specimen must, in the first place, be well washed and floated upon a glass slide with a considerable quantity of water, which must be allowed to

* On the Anatomy of the Liver of Man and Vertebrate Animals. —London: John Churchill, 1856.

flow off the slide very gradually. The specimen may then be allowed to dry under a glass shade, in order that it may be protected from dust. The drying should be effected at the ordinary temperature of the air, but it is much expedited if a shallow basin filled with sulphuric acid be placed with it under the same bell-jar.

Of solid organs, such as the liver and kidney, thin sections as well as portions from the surface should be preserved. Thin sections may be made with the ordinary scalpel or with Valentin's knife, if an extensive one be required. The surfaces of the section should be well washed, and it may then be mounted in one of the methods previously described.

LECTURE VIII.

Of the advantages of chemical reagents in microscopical investigation.—Instances of the use of reagents.—*Of the hardening properties of different solutions.*—Mr. Lockhart Clark's plan of preparing thin sections of the cord.—Method of rendering tissues hard and transparent.—Chemical reagents required by the microscopist.—Testing for carbonate and phosphate of lime, phosphate of ammonia and magnesia, sulphates, and chlorides.—Of taking notes of microscopical observations.—Of the importance of making sketches.—Of taking photographs of objects in the microscope.—Of making observations upon objects in the microscope.—Of drawing inferences from observations.—*Fallacies to be guarded against in microscopical investigation.*—Errors of observation.—Of the commencement and termination of tubes.—On the difficulty of seeing structures from their transpareney.—Fibres and membranes artificially produced by action of reagents.—A fibrous appearance produced in structureless membrane.—Collection of oil globules appearing as if within a cell-wall.—On the accidental presence of extraneous substances.—Conclusion.

Of the Advantages of Chemical Reagents in Microscopical Investigation.

Gentlemen,—We were considering not long since the influence which the refractive power of the medium in which any

structure was examined exerts upon its appearance in the microscope. We have now to discuss the advantages derived from the chemical action of certain solutions upon various specimens. This part of the subject is most important, and it is perhaps of all the various branches of microscopical research, that from which the greatest advantages may be expected to result,—an investigation which will certainly reward all who earnestly devote themselves to its study. It is probable that great changes will take place in our views of the nature of many minute structures when chemical analysis shall be more intimately associated with microscopical inquiry. Each branch of research will derive assistance from the other, and by the aid of both the exact nature of various structures may be ascertained which cannot be elucidated by either inquiry separately.

Besides the ordinary uses to which they are applied, chemical reagents are useful in removing certain components of a structure which interfere with the examination of other constituents, in altering the character of certain tissues without dissolving them, as for instance by increasing their transparency or opacity, or in modifying the physical structure of textures in such a manner as to render it more convenient to cut sections or to perform other mechanical operations necessary for the demonstration of their structure.

Instances of the Use of Reagents.—Various insoluble saline materials not unfrequently prevent us from seeing the anatomical elements of which a tissue is composed. A knowledge of the nature of these often enables us very easily to remove them. Supposing, for instance, the saline matter consists of carbonates or phosphates of lime or magnesia, we have only to add a drop of dilute acid which dissolves them completely.

The action of acids and alkalies is often very valuable in rendering structures transparent, which are too opaque for examination in the ordinary state. If I take a portion of this tendon, composed of white fibrous tissue which is very opaque in its ordinary state, and immerse it in acetic acid, or in a dilute solution of potash or soda, it soon becomes clear and transparent, and if the operation be conducted with certain precautions, many of its original characters may be brought back by subsequently neutralizing the acid or alkali.

The cell wall which in this diagram is represented as being too opaque to enable us to see the nucleus in the interior of the cell, in the adjacent one is represented as perfectly transparent with the nucleus well defined in its interior, a change which may be easily effected by either of the reagents which I just now alluded to. Albuminous textures generally may often be rendered very transparent by the action of acetic acid, or by the addition of a drop of dilute caustic potash or soda.

Of the Hardening Properties of Different Solutions.—The consistence of many tissues is so soft that it is absolutely impossible to obtain a thin section; while, by tearing off a small piece, the relations of the component parts is usually so much altered, as to render the specimen useless for the purpose of examination. In this case our only chance is to harden the texture by some reagent in such a manner that, although its microscopical characters are not altered, a thin section may be readily obtained.

It is even possible in some instances to render a soft tissue sufficiently hard to enable us to cut very thin sections, which may afterwards be restored to their former consistence by the complete removal of the hardening solution.

The nature of the solution employed for hardening a tissue will depend upon the character of the texture itself. Many tissues may be hardened in alcohol, others may with advantage be soaked in a weak solution of chromic acid, which contains a sufficient quantity of the solid acid to render the solution of a pale straw-colour. Various saline solutions are also sometimes employed for hardening tissues, but in consequence of the alteration they produce in the texture of the substance, they are not well adapted for many microscopical specimens. Boiling in water, is often resorted to for the same purpose. In this way very thin sections of such textures as muscular fibre may be obtained which may afterwards be rendered transparent by being soaked in syrup or glycerine, or by the addition of a little solution of caustic soda or potash.

The muscular fibre cells characteristic of *unstriped* or *involuntary muscle* may be rendered hard enough to permit their separation and isolation by being soaked for some days in dilute nitric acid, or in a mixture of nitric and hydrochloric acids (one part of acid to four of water). A solution of

sesquichloride of iron is also employed for hardening some tissues with advantage.

The hardening properties of the solutions which I have just referred to, depend essentially upon their power of coagulating albuminous substances, and in the majority of instances the coagulation is associated with a certain opacity quite incompatible with the satisfactory examination of the tissue by transmitted light, and as I have before hinted, it is absolutely necessary to render such a specimen transparent after the thin section has been obtained. It is well to bear in mind that before we can submit many soft structures to microscopical examination, we have to consider what chemical substances are likely to harden them in the most advantageous manner for cutting thin sections, and if by this process the section be made opaque we have next to consider further how its natural transparency may be restored. The chemical nature of the substance to be examined, its physical properties generally, its refractive power, and its chemical composition, are points which it is most desirable that every microscopic observer should be acquainted with before he commences any particular investigation.

Mr. Lockhart Clarke's Plan of Preparing Thin Sections of the Cord.—By a peculiar method of preparation my friend Mr. Lockhart Clarke has obtained most beautiful sections showing the arrangement of the nerve-fibres, and vesicles in the spinal cord and other parts of the nervous system. The results of his observations are recorded in the Philosophical Transactions for 1851, Part II. The cord is first hardened in acetic acid and alcohol, when excessively thin sections may be readily obtained with a sharp knife. These are then soaked in pure spirit which permeates the texture in every part, and drives out the acetic acid. It is then transferred to turpentine which expels the spirit, and lastly the sections are mounted in Canada balsam.

Method of Rendering Tissues Hard and Transparent.—This little embryo, the tissues of which have been rendered so transparent that the smallest ossific points can be seen in the temporary cartilages, serves to illustrate the value of chemical compounds to the microscopical observer. To dissect these bony points at so early a period, would be a work of immense

PLATE XXVIII.

Fig. 132.

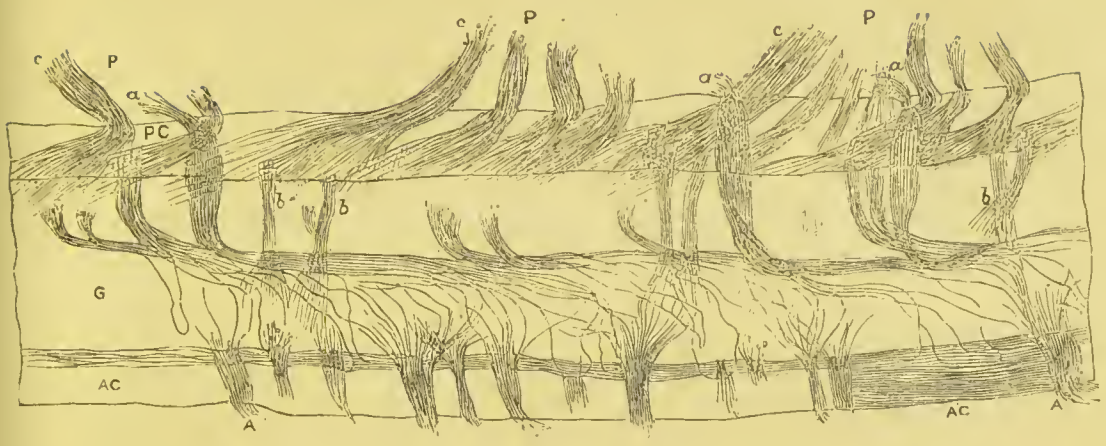


Fig. 133.



To illustrate Mr. Lockhart Clarke's method of making preparations of the spinal cord (p. 88).

Fig. 132. Longitudinal section through the spinal cord in the neck of a cat. PC. Posterior white column. AC. Anterior white column. G. Grey substance between the white columns. P. Posterior roots of the nerves, consisting of three kinds,—*a*, *b*, and *c*. A. Anterior roots of the nerves. AC. A portion of the anterior column, showing the arrangement of the longitudinal fibres.

Fig. 133. A transverse section through one-half of the lumbar enlargement of the cord, representing the course of the fibres of the roots of the nerves, and the transverse commissures through the grey substance. The vesicles have been omitted to prevent confusion. The fibres of the anterior roots, *i*, *i*, on reaching the grey substance, are seen to diverge and cross each other; and those of the posterior roots, are seen intersecting each other in every direction. [To face page 88.]

labor, but by merely soaking the whole organism in the solution all these points become beautifully distinct. The specimen is to be immersed in alcohol to which a few drops of solution of soda have been added, and allowed to remain in it for a few days. When the action has taken place, it is to be removed and preserved permanently in weak spirit. This preparation has been kept for nearly four years, and preserves its character unaltered. The principle of the action of the fluid may be explained thus, alcohol alone tends to coagulate albuminous textures and render them opaque, at the same time that it hardens them. The alkali on the other hand will render the tissues soft and transparent, and, if time were allowed, would cause their complete solution. These two fluids in conjunction harden the texture and at the same time make it clear and transparent. Many soft tissues may thus be hardened sufficiently to enable us to cut very thin sections.

Preparations of this kind show how much may be effected by the use of very ordinary chemical reagents. By this simple process, a minute dissection which would extend over days is avoided, the chance of losing some of the small ossific points is prevented, while the structures are displayed far more distinctly than could be done by the most careful dissection.

Doubtless there are many other fluids yet to be applied to the purposes of investigation of much greater value than the present one, and I strongly recommend you to take up this branch of inquiry and endeavour to discover new modes of preparing textures which shall render their minute structure clearly demonstrable.

Chemical Reagents Required by the Microscopist.—The number of the chemical reagents required is not great, but it is very important that the student should be familiar with a few of the reactions which indicate the presence of common substances, and which have the power of rendering certain textures more transparent or more opaque than they are in the natural state.

The most important reagents are the following :—

Ether and Alcohol.
Acetic acid,
Nitric acid, Chromic acid.

Solution of potash.
Solution of soda.
Ammonia.
Nitrate of barytes.
Nitrate of silver.
Oxalate of ammonia.
Iodine solutions.

These tests may be preserved in ordinary stoppered bottles, but I much prefer to keep them in small tubes with capillary orifices, from which only a drop, or a part of a drop, can be expelled when required. Several years since, I arranged all the ordinary tests I required for microscopical purposes in small bulbs which were drawn off to a capillary point. They were provided with glass and gutta percha caps. These bulbs, however, were somewhat inconvenient in consequence of not being made to stand upright, and Mr. Highley substituted for them, tubes with flat bottoms and ground glass caps. I will now try to fill one of these little vessels with the solution it is intended to contain. A little of the solution is poured into this small basin, the tube being inverted so that its orifice dips beneath the surface of the fluid. Heat being now applied to the body of the bulb, the air in its interior is expanded and partially expelled. As the bottle becomes cool, a certain quantity of the fluid rises up into its interior. Usually, however, it is not possible to introduce more than a few drops in this manner. The bottle is then removed and heated over the spirit-lamp until the drop of fluid in its interior is in a state of ebullition. While the steam is issuing violently from the orifice, I carefully plunge it again beneath the surface of the fluid. As the steam within condenses, the solution rises up in the interior, and would completely fill the little bottle if it were maintained in this position, but when it is about three parts full I remove it from the fluid. If I were to fill it completely it would be difficult to expel the fluid when required. A certain quantity of air, therefore, is allowed to remain within the bottle, and being expanded by the warmth of the hand, the quantity of fluid required can be driven out at pleasure. In this manner fluid may be introduced; but Mr. Highley has made a further improvement by arranging the capillary neck in the form of a tubulated stopper,

PLATE XXIX.

Fig. 134.

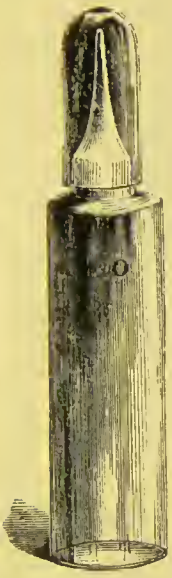


Fig. 135.



Fig. 136.



Fig. 137.

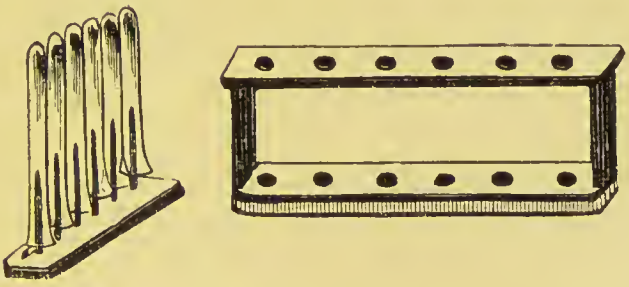


Fig. 138.

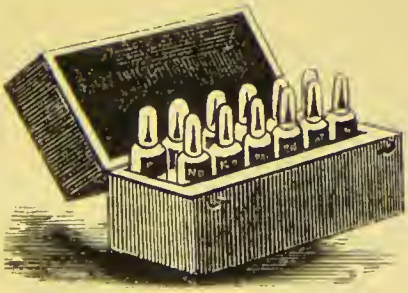


Fig. 139.



Fig. 134. Small tube with capillary orifice for microscopical testing (p. 90).
Fig. 135. Tube with India-rubber, *a*, tied over upper extremity, to remove small quantities of test solutions from bottles. *b*. Ground, to fit into the neck of the bottle. *c*. Orifice.
Fig. 136. Bulb with capillary orifice (p. 90).
Fig. 137. Test tubes, rack, and drainer.
Fig. 138. Box with test solutions in bottles with capillary orifices.
Fig. 139. Crystals of common salt.

[To face page 90.]

by the removal of which fluid can be introduced as in filling an ordinary bottle.

For microscopical purposes these little bottles possess many advantages over the ordinary stoppered bottle.

In the *first* place, a most minute quantity of the test can be obtained without difficulty, and there is no chance of too much escaping.

Secondly, there is no danger of the reagent becoming spoilt by the introduction of various substances from without. If an ordinary stoppered bottle be used, a drop of the fluid must be removed with a pipette or stirring rod, but if these should not be quite clean, foreign substances may be introduced, and the reagent spoilt for further operations. Carelessness upon this head will lead to the greatest inconvenience, and give rise to serious mistakes.

Thirdly. Testing by means of these little bottles can be conducted in a very short space of time, and they possess the advantage of being packed in small compass.

Testing for Carbonate and Phosphate of Lime, Phosphate of Ammonia-and-Magnesia, Sulphates and Chlorides.—Now, suppose I wish to ascertain the nature of the substances composing this earthy matter. I take a small portion, about the size of a pin's head, place it upon this slide, and cover it lightly with a piece of thin glass. Next, I expel a drop of *nitric acid* (by warming in the hand the bottle with the capillary neck) close to the thin glass. The acid soon reaches the sediment, and now, one may observe the disengagement of a few bubbles of gas, which are, as it were, temporarily pent up by the thin glass, and prevented from escaping. If there should be any doubt of the action of the acid, we may resort to examination in the microscope, when, if there be very few bubbles, they may be detected. In this deposit *carbonates* are present.

The acid solution is now neutralized with *ammonia*. A faint flocculent precipitate is produced. After this has stood still for a few minutes it is covered with thin glass and examined under the microscope. It will be seen to consist of amorphous granules (Phosphate of Lime) and small crystals, which, if allowed to stand long enough, will take the form of triangular prisms (Phosphate of Ammonia and Magnesia).

If we wish to ascertain the presence of sulphates, a little of

the nitric acid solution is treated with *nitrate of Barytes.* An amorphous precipitate of Sulphate of Baryta, insoluble in strong acid and alkalies, takes place, if sulphuric acid be present. The presence of chlorides is detected by the addition of a little *nitrate of silver* to a drop of the solution of the deposit in weak nitric acid. The white precipitate of chloride of silver is insoluble in *nitric acid,* but it is dissolved by *ammonia.*

These will serve as examples of the method of detecting the presence of different substances in a very minute quantity of matter. The indications obtained in this manner are quite as valuable, and may be relied on with as much certainty, as if we were provided with a very large quantity of material to work upon; in a single drop of a composite solution, the presence of several different acids and bases may be detected.

Of Taking Notes of Microscopical Observations.—I must now say a few words upon the subject of taking notes of observations. It is of great importance to acquire the habit of describing in words, the appearance of objects under the microscope. This is probably not so easy as would at first be supposed, although many persons are able to describe what they see, much more correctly, and with greater facility, than others. Accuracy in describing microscopical specimens can only be acquired by practice, and I think it a most excellent rule to take notes of the appearances of every object submitted to examination. The time is well spent, and everything so described is retained in the memory. The notes should be short, and should consist of a simple statement of points which have been observed. Inferences should be carefully avoided, and nothing should be stated without the observer being thoroughly satisfied of its accuracy. If he is not quite certain of any observation he should express his doubts, or place a note of interrogation after the statement. The use of indefinite terms should be avoided as much as possible, and whenever any particular word is used a definite meaning should be attached to it. Much confusion has arisen from the use of terms which have not been well defined. Thus the word "*granule*" by many authors is applied to a minute particle which appears as a small speck even when examined by the highest powers, as well as to a small body with a perfectly clear centre, and with a well-defined sharp outline, which would be more correctly

termed a small "*globule*." So, again, the term "*molecule*" has been employed in some cases synonymously with "*granule*," but it would obviously be wrong to speak of a small globule as a molecule. It seems to me very desirable to restrict the terms "granule" and "molecule" to minute particles of matter which exhibit no *distinct structure* when examined by the highest powers at our disposal, and the term "globule" to circular or oval bodies of all sizes which have a *clear centre*, with a *well-defined dark outline*. Other examples of the use of insufficiently-defined terms might be pointed out. If an observer makes use of a term which is generally employed without any definite meaning being attached to it, he should describe at length the meaning which he assigns to it, and should, of course, use it only in this one sense.

Exactness of Description should always be aimed at, and we must remember that with a little trouble this exactness may be obtained with the use of a small number of words. That appearance of precision which is often attempted by employing long useless descriptions cannot be too much condemned. So, also, the practice of some, of describing every object in the field of the microscope without the smallest knowledge of any one of them, has been the cause of much ridicule, and has brought microscopic observation into great disrepute. Some have thought to gain the credit of being accurate observers by carefully measuring every object they see in every diameter, and putting down in numbers the results of this useless ceremony.

Such reports show that the author is thinking more of himself than his subject. He attempts to acquire a character of extreme minuteness of observation, instead of striving to advance the real interests of the science which he professes to serve—and instead of endeavouring to excite in the mind of the reader a desire for more extended knowledge and a wish to take part in a similar investigation, he perpetually gives undue prominence to himself. He who feels a real love for his subject will try all he can to enlist others in the same cause; he will try to remove all difficulties of investigation, and endeavour to express what he has learned himself in language which shall be intelligible to all. A certain mysterious air pervading the description of an observation,—an evident

desire to coin new words,—and exaggerated statements of the importance of the facts observed, are quite misplaced where all should be clear, simple, and intelligible to every one,—and too often show indifference to the subject on the part of the author, and a want of consideration towards unlearned readers. Nothing, I believe, has been productive of so much pain and sorrow to earnest men who have devoted long lives to the prosecution of different branches of natural science, or retarded the real progress of scientific enquiry, more than that affectation of precision, and minute verbose and pompous style of description, which has been fashionable among some microscopists, and which pervades the writings of several authorities in this imperfectly developed branch of investigation in the present day. All this is mere pretence, and not real, earnest, useful work,—distasteful to every scientific man and discouraging to every student. An extreme minuteness in description is by no means a proof of accuracy of observation. In this manner science becomes encumbered with unnecessary words, and earnest students are often intimidated when they commence investigations for themselves.

Of the Importance of Making Sketches.—Of the great importance of drawing I have already spoken. Even mere sketches in outline are of great value if the size has been correctly observed, and by appending to the drawing one of the small scales alluded to in my second lecture (page 23) the necessity for making measurements, and pages of uninteresting detail which would never be read, are avoided, and at the same time greater accuracy is obtained.

I think that lithographs afford the most accurate representations of microscopic structures, but woodcuts possess the great advantage of being inserted in the text, and many of them convey a good idea of the structures they are intended to represent. Elaborate woodcuts like copper-plate and steel engravings, are, however, very expensive. Engraving on stone possesses many advantages, and some of the most beautiful microscopic drawings which have been published lately have been engraved in this manner. The most beautiful illustrations of structure by Mr. Tuffen West are engraved upon stone, and many of the best German drawings are made in the same manner. For all ordinary objects the usual process of drawing

on stone with lithographic ink, and prepared chalk, will give the requisite delicacy. Lately I have copied a great number of objects upon prepared transfer paper by tracing them directly with the aid of the neutral tint glass reflector, drawing the outlines with ink, and shading with lithographic chalk This drawing is then at once transferred to the stone, from which copies may be printed off. This process is not expensive ; it is necessarily very accurate and easy of execution, but the drawings appear rather rough, and are certainly not beautiful as works of art ; still they give a good idea of the appearances observed, and, being drawn directly from the object itself, they must be accurate. Several of these are placed on the table, with the scales of measurement also delineated as I have before mentioned.

Of Taking Photographs of Objects in the Microscope.—Of late years many objects have been photographed very successfully. The advantages of such a plan are so obvious that I need not occupy your time in recounting them. In the hands of Mr. Delves, Mr. Shadbolt, Mr. Julius Pollock, and others, very satisfactory results have been obtained, and we may hope that in time perfectly good copies will be made in this manner.

In arranging the microscope for taking photographs, the camera requires to be much lengthened. There should be a distance of two feet between the object glass and the position of the sensitive plate. This increased length may be obtained by having a brass tube, about two inches in diameter, arranged to screw into the ordinary position of the lens of the camera. The further extremity of this tube is adapted to receive either the object-glass itself, or that part of the body of the microscope into which the latter fits. The ordinary microscope stage may be used, or a stage may be adapted to the end of the tube as in the microscope camera of Mr. Highley, or the stage upon which the object is placed may be supported upon a separate stand. The foci of the chemical and visual rays are not coincident in the ordinary object-glasses, consequently several experiments have to be made in order to find the exact focus of the chemical rays, and we must take different pictures until one is found to be in focus. The degree in which the fine adjustment screw has been turned to produce the best effect is then noted.

Negative photographs of microscopic objects can only be taken by sunlight, and the light must either be thrown upon the object from a plane mirror, or condensed upon it by means of a large plano-convex bull's-eye condenser. Care must, however, be taken that the object is not subject to the intense heat produced in this operation, longer than absolutely necessary, especially if mounted in fluid or Canada balsam.

The whole apparatus should be mounted upon a stiff board which can be inclined conveniently at any angle.

The plate is prepared in precisely the same manner as for taking ordinary photographs. The time of exposure must be found by experiment. From ten to twenty seconds will be sufficient in a very good light. The collodion made by Mr. Thomas takes capital pictures, but I have reason to think that the collodion prepared by my friend Mr. Hardwich, of King's College, will be found very advantageous for microscopical purposes.

Mr. Delves, of Tunbridge, has produced some very beautiful microscope photographs, many of them being very highly magnified. Indeed, some representations of the navicula far exceed in delicacy and finish the most perfect engravings. It is impossible that such minuteness can be obtained by the hand of the artist as may be ensured by this simple process.

Many anatomical specimens, however, cannot be copied by photography, especially if they be very thick. The yellow colour of the tissue in most instances precludes the possibility of making a photograph of it, as the transmission of the light is so much interfered with; and this is an especial objection in the case of injections viewed as transparent objects, for the tissue intervening between the vessels, is often so yellow that these intervals in the photograph become as dark as the vessels themselves. My friend Mr. Julius Pollock has nevertheless succeeded in obtaining for me some very tolerable copies of injections of the distribution of the ducts in the liver. By practice, doubtless, many improvements in the process of taking photographs of microscopic objects would be effected.

When only few copies of a work are required, the researches may be very cheaply illustrated by taking photographs of drawings. A large drawing of the object must first be made in the manner described in page 21. From this a negative

PLATE XXX.

Fig. 140.

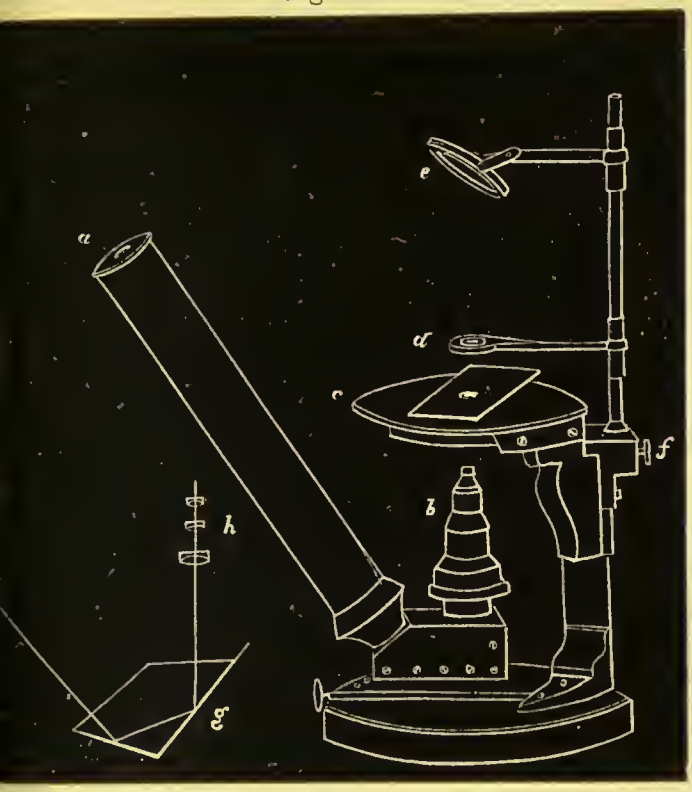


Fig. 141.



Fig. 142.

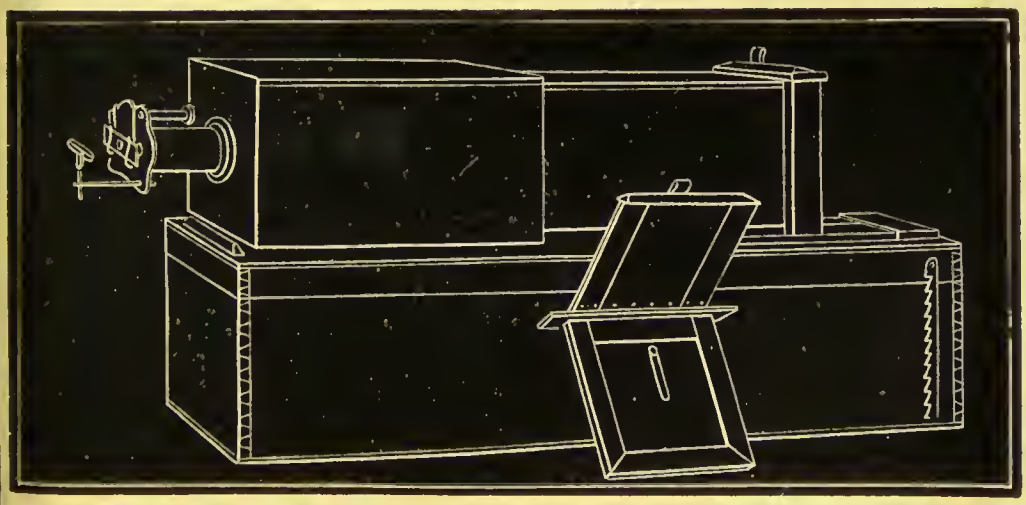


Fig. 140. Inverted microscope of Dr. Lawrence Smith, for examining objects in acid solutions, corrosive fluids, &c. *a*. Tube of microscope with eye-piece. *b*. Object glass over the box which contains the prism resembling that represented at *g*. *c*. Stage with slide upon it. *d*. Support on which polarizing apparatus or condenser may be placed. *e*. Mirror. *f*. Screw by which the stage is elevated or depressed in focussing. *g*. Prism showing the direction in which the rays of light passing through it are refracted. *h*. Position of achromatic object glass.

Fig. 141. Apparatus for examining objects while exposed to the action of a high temperature.

Fig. 142. Stage and mirror adapted to ordinary photographic camera for taking photographs of microscopic objects as arranged by Mr. Highley (p. 95).

[To face page 96.]

reduced to the proper size is taken, from which any number of copies may be obtained. In this manner I have illustrated my memoir on the anatomy of the liver with upwards of sixty illustrations. When many copies of a work are likely to be required, this mode of illustration is not applicable, as the original cost of engraving would soon be covered ; but when only a *few* copies of a *great number* of drawings are wanted this plan possesses decided advantages.

Of Making Observations upon Specimens in the Microscope.— The eye of the observer requires much careful education before he is able to appreciate fully the character of the structure which he is examining. If, upon examination, a specimen does not appear to him to justify the description or delineation which some observer has given of a similar structure, he must not too hastily infer that the author has been recording the results of his imagination, rather than observed facts. We must remember that the conclusions which have been arrived at are probably the result of a very long and patient investigation, deduced from examining a specimen under very different circumstances, after the application, perhaps, of various chemical reagents, and after ascertaining the effect of different refractive media. From the remarks which I made in my fifth lecture you will have formed some idea of the many different operations which are necessary to demonstrate conclusively, the anatomy of a single tissue. You must not, therefore, be too hasty in deciding upon the nature of an object in the microscope ; neither must you infer that what you have not been able to see, does not therefore exist.

Some fall into an error of the very opposite description, not less detrimental to forming habits of correct observation. Led away by their imagination, they think they see everything which has been delineated or which they have heard described ; the observations of authors are confirmed in expressions closely resembling the original and thus, in point of fact, their own testimony is brought forward, though not directly by themselves, a second time in favor of their original doctrines without any real confirmation of the accuracy of their views being advanced. In this manner errors have been propagated and strengthened to an extent almost incredible, and years of laborious investigation have been

spent in overthrowing statements which had never resulted from actual observation in the first instance.

Of Drawing Inferences from Observations.—No one engaged in the pursuit of any branch of natural science is more tempted to be led into too hasty generalization than the microscopical observer. It is his duty, therefore, to avoid drawing inferences until he has accumulated a vast number of facts to support the conclusions to which he has arrived. True generalizations and correct inferences promote the rapid advancement of scientific knowledge, for each new inference clearly forms the starting point of a fresh line of investigation: but we must remember that on the other hand, every false statement regarded as an observed fact, forms a terrible barrier to onward progress, since, before the slightest useful advance can be made it is necessary to retrace our steps, it may be for a considerable distance, before we can hope to recommence our onward course. Again, a much greater amount of evidence is always required to overthrow a false conclusion than is sufficient to propagate the error; and there can be no task more unsatisfactory than to be compelled to subvert the opinions and deductions of others.

Scientific enquiry ought continually to advance, and we should be able to extend our researches from the point where they have been left by our predecessors, adding successively to what they had discovered; but the observations which we owe to them should not require correction. In not a few instances must we feel the highest respect for the careful observations of the older observers, and I fear it must be reluctantly confessed that many of our modern researches are not carried out with the same conscientious care as theirs and are likely to be but short lived.

Now there are many mistakes which an observer is very likely to commit unless he be warned of their nature in the first instance. Some of these it will be well for me to advert to as briefly as possible.

Fallacies to be Guarded against in Microscopical Investigation.

Many mistakes have arisen in consequence of sufficient care not having been taken to prevent the introduction of various

substances by accident. The most scrupulous cleanliness must always be observed in microscopical examination, and any foreign particles which may have accidentally come into contact with the preparation must be carefully removed before it is mounted.

The plan of proceeding will depend much upon the nature of the texture and that of the foreign matter. Mere dusting with a camel's-hair brush, washing in a stream of water, or picking out the object with needles, are simple plans which are often efficient in a general way, but in some cases other processes are required.

Errors of Observation.—Every observer must be careful to avoid making erroneous observations. One is liable, not only to draw false conclusions from observations, but the observations themselves are not unfrequently erroneous. I propose to draw your attention to a few of what appear to me frequent sources of difficulty and doubt even to the most experienced.

Of the Commencement and Termination of Tubes.—The mode of commencement or termination of certain vessels or tubes have long been sources of dispute among observers. There are not a few instances where positive statements have been made that certain tubes commenced by cœcal or blind extremities; while contradictions equally positive have been advanced by others, who have held that the tubes commenced as a network, and presented no blind extremities whatever. It would be supposed by many that this point might be placed beyond all doubt by injecting the tubes with some coloured material. But this is not so. Injection will frequently run up to a particular point in the minute vessels, while no force which could be applied could drive it further onwards. Here, therefore, it accumulates, and often to a very considerable extent, the portion of the tube above the constriction being considerably dilated by the pressure which has been applied. Under these circumstances it is impossible to trace the further continuity of the vessel, owing to the extreme transparency and delicate nature of the tissue of which its walls are composed. Indeed, these may be quite invisible in an unprepared specimen. The observer is thus led into the error of supposing that such tubes terminate in blind extremities, whereas they

may really form a network with large meshes, or they may be continuous with other structures beyond ; and that which was taken for the termination or commencement of the tube may really be nothing more than a bulging in a central part of its course. In many thin sections of the kidney an appearance as if the tubes terminated in free blind extremities is produced in consequence of the convoluted portions of a tube lying in such a position that the recurved portion is immediately beneath the most superficial part of the tube. From a mere examination of the specimen it would be impossible for any one to say that this was not the case. In such instances the real disposition of the parts is only to be made out by a careful examination of the structure under different circumstances and prepared in various ways. Thus the idea that the tubes end by blind extremities may be shown to be quite inconsistent with the appearances observed in one particular mode of examining the texture. I am unable, however, to devote much time to the consideration of this part of my subject, or I might review the various methods in which a tissue is examined, and show how by a consideration and comparison of the different facts observed, one is enabled at length to embody the results arrived at in several different inquiries, and form an idea of the real structure of the part. The remarks which I made in a former lecture were, no doubt, sufficient to direct your attention to these important points (page 48).

On the Difficulty of Seeing Structures from their Transparency.—Another fallacy arises from the great transparency of certain structures. Oftentimes a membrane may appear perfectly clear and transparent when in reality it is covered with a delicate layer of epithelium, which only becomes visible by being immersed in some special fluid or treated with some particular chemical reagent. On the other hand, there are instances in which an appearance resembling that produced by the presence of a cellular investment is perceived where no cells whatever exist. A peculiar corrugated state of uninjected capillaries, and the cells in the walls of the capillary vessels themselves, sometimes give rise to these mistakes. Basement membrane, from its extreme delicacy and transparency, is often only recognized by the folds into which it is thrown, or by the debris and granular matter which is acci-

dentally adherent to it. Sometimes it becomes visible when immersed in a slightly-coloured solution, instead of in perfectly pure water.

Fibres and Membranes Produced by the Action of Reagents Artificially.—On the other hand, by the action of reagents a fibrous appearance is sometimes produced which, without care, may be mistaken for a portion of the structure under examination.

The addition of acetic acid to many preparations frequently produces a swelling of the tissue, with the elevation of a clear membranous structure, which might be termed basement membrane, but which has really been formed in this manner.

A Fibrous Appearance Produced in Structureless Membranes.—Clear, transparent, and apparently structureless membranes when pressed, torn, and twisted, has a fibrous appearance, and delicate vessels, whose coats are perfectly transparent when pressed and collapsed, may be very easily mistaken for a form of fibrous tissue. If any doubt exist in such a case, it may always be cleared up by injecting the capillaries of the part with a clear transparent material, like plain size, when, if the fibrous appearance were not real it will be lost; while if fibres really existed, they would still be visible. The presence of capillary vessels in a structure has been entirely overlooked in consequence of their being collapsed and shrunken, in which state they have been described as fibrous tissue.

Collection of Oil Globules Appearing as if within a Cell.—Oil globules in fluid not uncommonly form small and nearly spherical masses or collections, which become covered with a certain quantity of mucus or viscid matter and granules, originally contained in the fluid, so that the little intervals between the minute oil globules become filled up; the outline of the mass is perfectly clear, and sharp, and well defined, and from mere ocular examination it is impossible to say that the oil globules are not enclosed in a cell-wall. A consideration of the circumstances under which such structures have been met with, will often assist us materially in determining their real nature.

On the Accidental Presence of Extraneous Substances.—I believe, however, that the most common errors may be traced to the accidental presence of substances which are not familiar

to the observer, and which are mistaken by him for objects which he is looking for.

When we consider how minute many of the structures rendered evident to the eye by the microscope, are, we shall scarcely wonder that many light substances are liable to come in contact with the specimen which is under examination, and if we are not familiar with their characters, they may give rise to great difficulty. The cotton or flax fibres from the cloth, starch globules which adhere to the thin glass (for the small pieces are usually kept in starch) portions of feathers, various kinds of hair and oil-globules are among the substances which are most frequently met with in examining different structures, and I need hardly say that their presence is purely accidental. To convince you that I am not giving you needless caution upon this head I may mention that in a well known and otherwise highly valuable publication, a drawing of what is evidently a portion of feather is described as a representation of *lymphatic vessels*,—vegetable hairs are described as *nerve fibres*, and several other errors equally unpardonable occur. Now such mistakes could only arise from utter ignorance of the characters of some of the commonest objects with which every observer ought to be very familiar. I would very strongly recommend every one to study the characters of all these substances before he attempts to make any original observations. He is sure to meet with them from time to time, and the sooner he is well acquainted with their characters the better.

The following should be very carefully examined :—

Oil globules, milk.
Potato, wheat and rice starch, and bread crumbs.
Portions of feathers, worsted.
Fibres of flax, cotton, and silk of different colors.
Human hair, cat's hair, hair from blankets.
Fibres of wood swept from the floor, fragments of tea-leaves, hairs from plants, vegetable cellular tissue, and spiral vessels.
Particles of sand.

In the examination of deposits from fluid we must bear in mind the possibility of the admixture of a small quantity of one deposit with another by the pipette used for examina-

PLATE XXXI.

Fig. 143.

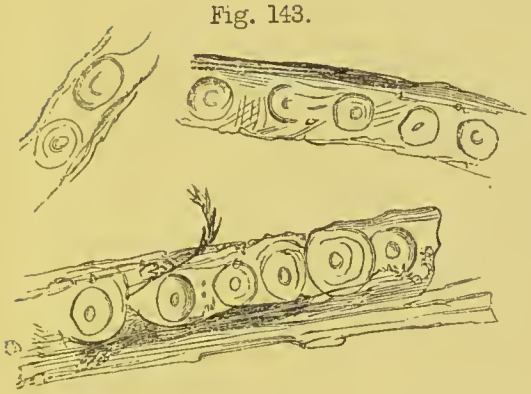


Fig. 144.

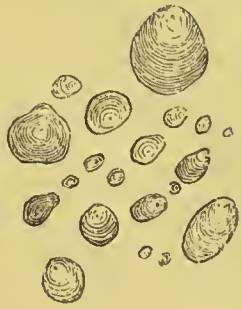
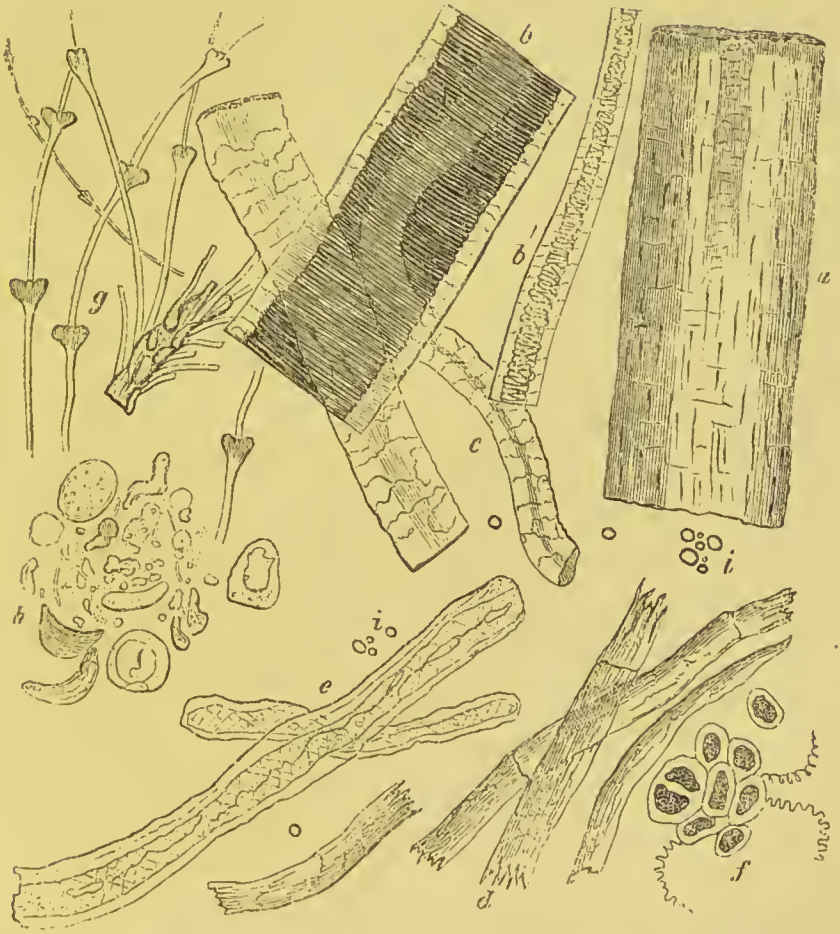


Fig. 145.



Substances which are often accidentally present in microscopical preparations.

Fig. 143. Fibres of deal swept from the floor (p. 102).
Fig. 144. Globules of potato starch (p. 102).
Fig. 145. *a*. Fragments of human hair. *b*. Cat's hair. *c*. Hair from blanket. *d*. Fibres of flax. *e*. Fibres of cotton. *f*. Fragments of tea-leaves, showing cells and spiral vessels. *g*. Portions of feathers. *h*. Bread crumbs, showing wheat starch partly altered by baking and maceration. *i*. Free oil-globules (p. 102).

[To face page 102.]

tion, and in this simple manner much difficulty and confusion may be caused to the microscopist. The pipette should be well washed immediately after it has been employed and the water which is used should be very frequently changed. In taking fluids from different bottles and other vessels the possibility of introducing various substances must be borne in mind.

With these remarks, then, I must bring this short course of lectures to a close. Before we part, however, let me assure you it will always be a source of pleasure to me to be able to afford you any help in my power, and I trust, that from time to time you will give me the advantage of learning any processes you may have found by experience to be useful in the course of investigation,—for in microscopical inquiries we are all learners, and may be of mutual assistance to each other.

In conclusion, let me cordially thank you for the attention you have given to the subjects which I have brought under your notice, and if I have been able to give you any hints, or have offered any suggestions which may afford you help in your microscopical labors it will be an additional gratification to me.

TABLE I.

Arrangement of the Instrument for Observation.—Drawing, and Measuring Objects.

1. Arrange the microscope for examining objects by transmitted light.

2. Examine the objects upon the slide[1] with the inch, and afterwards with the quarter of an inch object-glasses, using first the shallow, and afterwards the deep eye-piece.

3. Arrange the mirror in such a manner that the rays of light may pass through the object in a direct course or obliquely.—p. 7.

4. Examine the same object under the quarter of an inch object-glass with the achromatic condenser, and afterwards without the use of this instrument.—p. 17.

5. Draw upon paper some of the objects[2] on the slide.—p. 20.

   a. Judging of the size by the eye alone.
   b. By placing the paper on a level with the stage.
   c. With the aid of the neutral tint glass reflector.

6. Ascertain the diameter of the objects upon the slide,[3] using the inch object-glass and stage micrometer divided to 100ths of an inch, with the aid of the neutral tint glass reflector.—p. 23.

7. What is the magnifying power of the two French and English object-glasses on the table.[4]—p. 24.

   a. With the shallow eye-piece.
   b. With the deep eye-piece.

8. Measure the angles of the crystals[5] upon the slide.—p. 23.

[1] Scales from the wing of a butterfly.
[2] Tracheæ from a caterpillar.
[3] Fragments of human hair.
[4] French quarter and one inch.—English quarter and one inch.
[5] Crystals of cholesterine.

TABLE II.

Examination of Objects by Direct or Reflected Light, Transmitted Light, and Polarized Light.

9. Examine the objects upon the slide[1] and carefully note the different appearances produced by examining them,

    1. *By reflected light* as opaque objects, employing

        a. The bull's-eye condenser.—p. 14.
        b. The Lieberkuhn and a stop.—p. 15.

    2. *By transmitted light*, employing,

        a. Direct rays.
        b. Oblique rays.

    3. *By polarized light*,

        a. Employing the polarizer and analyzer only.
        b. After placing beneath the objects a plate of selenite.

10. Examine some of the same crystals in different media, as described in p. 57.

    a. In air.
    b. In water.
    c. In turpentine, oil, or Canada balsam.

11. Examine the different appearance of the globules of potato-starch under the same circumstances.

12. Notice the microscopical characters of air-bubbles and oil-globules,[2] and examine them by reflected and by transmitted light.—p. 68.

[1] Spherical crystals of carbonate of lime.

[2] Small air-bubbles can be obtained by shaking a little gum-water in a bottle. A drop may then be placed upon a glass slide. *Milk* affords oil-globules in abundance.

TABLE III.

On Making Cells for Preserving Microscopical Specimens.

13. Make a paper cell and attach it to the glass slide.—p. 40.

14. Make a thin cell with the aid of marine glue, and another with tinfoil.—p. 41.

15. Make some square thin cells of Brunswick black, and some circular cells with the aid of Mr. Shadbolt's apparatus. —p. 41.

16. Cut some squares of thin glass with the writing diamond. —p. 41.

17. Cut some circular pieces of thin glass, using the brass circles.—p. 41.

18. Make some thin glass cells in the manner directed in page 43, and when complete grind the upper surface upon the emery slab.

19. Cut with the glazier's diamond some slips of glass, three inches by one inch, for slides.

20. Make a cell of thick glass in the manner described in pages 43, 44.

21. Make a deep cell of gutta percha. The gutta percha must be softened in hot water and then moulded upon some object the size of the required cell.

TABLE IV.

On Making Minute Dissections.—Cutting Thin Sections of Tissues for Microscopical Examination.

22. Trace the nerves in the portion of tissue on the table. Pin it out on a loaded cork, and dissect it beneath the surface of water with the aid of a strong light condensed upon it by the large bull's-eye condenser, in the manner directed in page 53.

23. Cut some very thin sections of the different soft tissues upon the table.
    a. Using the scissars.
    b. Using the double-edged knife.
    c. Using Valentin's knife.

    All these instruments must be well wetted before the section is removed.—p. 54.

24. Place some small pieces of tissue in the compressorium and dissect them under the microscope in the manner described in page 56.

25. Make some thin sections of wood with the aid of the section cutter alluded to in page 55.

26. Place some of the sections of pith or bone in thin cells, cover, them with thin glass, and let them be preserved as dry objects.—p. 61.

27. Ascertain the effect of the different preservative solutions upon the appearance of the sections in the microscope. —pp. 34, 38.

28. Place some of the sections which have been allowed to soak for half an hour in the fluid in which they are to be preserved, in thin glass cells, and apply the thin glass cover, observing the precautions detailed in page 63. Remove the fluid outside, and anoint the edge with Brunswick black, which must be applied with a small brush.

29. Make a thin section of the injected tissue on the table and preserve it in gelatine and glycerine.

30. Dry another section and mount it in Canada balsam.—p. 66.

TABLE V.

Kidney.—Muscular Fibre.—Pig's-skin.—Pith.—Wood.—Spiral Vessels.—Vallisneria.

31. Make thin sections of the sheep's kidney upon the table, and after washing them, subject them to examination with the inch, and afterwards with the quarter. Some may be examined in water and others in glycerine. One section should be mounted in the mixture of gelatine and glycerine.—p. 38. Observe the different characters of the tubes in the central and in the cortical portions of the organ, and endeavour to make out the following structures:—*Epithelium, basement membrane of the tubes, Malpighian bodies, and capillary vessels lying between the tubes.* The arrangement of the vessels may be satisfactorily demonstrated in an injected specimen.—Table VII.
32. Take a very small fragment of the muscular fibre of the skate or eel, and after tearing it up with needles, moisten it with water, and cover it with thin glass. Endeavour to find elementary fibres in which the tube of *sarcolemma* remains entire while the *sarcous* tissue within is ruptured.—p. 48.
33. The portion of pig's-skin on the table has been allowed to dry by exposure to the air. Thin transverse sections are to be removed with a sharp knife, and subsequently moistened with water. In this manner a very thin section may be obtained, which soon regains its normal appearance. It may be mounted in any of the preservative fluids before alluded to.
34. Cut thin sections of the dried cornea and sclerotic of the eye; soak them in a drop of water for twenty minutes or more, and examine them first with an inch object-glass and afterwards with a quarter.
35. Cut a thin section of the pith of the rush and examine it as a dry object; afterwards place it in fluid. Observe the air within many of the cells.
36. Demonstrate the circulation in the cells of vallisneria spiralis.—p. 65.
37. Wash some pieces of the sea-weed in plain water, and preserve some of them in glycerine, and others in solution of chloride of calcium.—p. 65.

TABLE VI.

Making Thin Sections of Bone, Hair, and Mounting them in Canada balsam.—Mounting Different Parts of Insects.—Separation of Deposits from Fluids.

38. Cut some thin sections of bone with the saw and grind them to the required degree of tenuity between the hones.—p. 55.

39. Upon microscopical examination they will be found covered with numerous scratches which must be removed by rubbing the sections upon a dry hone, and afterwards upon a piece of plate glass.—p. 56.

40. When the sections of bone are sufficiently smooth, mount one of them at once in balsam, and treat another section with turpentine before immersing it in the balsam. Compare the different microscopical characters of these two specimens.—p. 67.

41. Cut some thin transverse and longitudinal sections of hair, and examine them under the quarter of an inch object-glass. These may be washed in water and mounted in Canada balsam.—p. 55.

42. After drying several portions of the insects in the water-bath (claws, antennæ, wings, eyes, spiracles) moisten them with turpentine and mount them in Canada balsam.—p. 67.

43. After the deposit suspended in the fluid in the conical glass has subsided,[1] a portion is to be removed with the pipette and placed in a cell, or in the animalcule cage for examination.—p. 69.

44. The fluid may then be allowed to evaporate spontaneously or by placing the slide under a bell-jar over sulphuric acid, and the residue mounted in Canada balsam.

45. Subject some of the infusoria in the specimen of water on the table to examination with a quarter of an inch object-glass.[2]—p. 70.

[1] Small marine shells, sand, &c.

[2] Water containing portions of vegetables which had been kept for several day .

TABLE VII.

On Injecting with Opaque and Transparent Materials.—Prussian Blue Fluid for Injection.

46. Arrange the injecting apparatus conveniently (p. 80) and proceed to inject the artery supplying the eye-ball of the ox's eye on the table with size and chromate of lead.—p. 75.

47. *Eye.*—Introduce the pipe into the vessel running close to the large optic nerve, and tie it carefully, observing the precautions detailed in page 81. The eye must be allowed to remain in warm water until warm through, and the injecting material prepared in the manner described in page 75; it is to be mixed with melted size and strained immediately before use. When the injection is complete the eye is to be placed in cold water. Should it become very much distended by the accumulation of the injection within it, a puncture may be made in the cornea, which will permit the escape of the aqueous humour, and then the vessels may be more completely injected.

48. Prepare some Prussian blue injecting fluid.—p. 78.

49. *Frog.*—Insert an injecting pipe into the aorta of the frog in the manner described in page 81, and slowly inject the fluid.

50. The specimens having been completely injected portions may be submitted to microscopical examination.—p. 84.

51. The globe of the eye may be opened and portions of the *ciliary processes* situated behind the iris; of the *retina* (the most internal of the membranes within the globe); and of the *choroid*, (external to the delicate retina); after being carefully washed in water, may be submitted to examination in fluid with the inch object-glass.

    The ciliary processes and the choroid require to be well washed in order to remove the black pigment with which they are covered.

52. Portions of the lung and intestines of the frog may be removed, and after being well washed, may be submitted to examination. These are to be examined by transmitted light, and may be placed in glycerine. The inch object-glass should be employed in the first instance, and afterwards the quarter.

TABLE VIII.

Of the Use of Chemical Reagents in Microscopical Investigation.

53. Test the powder on the glass slide for the presence of carbonate,[1] using the precautions detailed in page 91.

54. The solution[2] is to be diluted and tested for sulphates, phosphates, and chlorides.—p. 91.

55. Make some crystals of common salt.

    a. By evaporating a solution rapidly to dryness on a glass slide.

    b. By allowing the solution to evaporate slowly until crystals form, when a thin glass cover may be applied and the crystals subjected to microscopical examination.

56. Fill one of the little bottles with capillary orifices, with acetic acid.

57. Examine some of the white fibrous tissue[3] under a quarter, before and after the addition of a drop of acetic acid.

58. Ascertain the effect of a solution of caustic soda upon the cells on the slide.[4]

59. Describe the microscopical characters of the structures upon the glass slide,[5] and sketch roughly their most important characters.

60. What is the nature of the substances forming the deposit in the glass.[6]

[1] Chalk.

[2] Sulphate of soda, phosphates of lime, and ammonia and magnesia, and common salt dissolved in water to which a few drops of nitric acid have been added.

[3] The white tendon of a musele of any small animal, as a mouse, &e.

[4] Cuticle.

[5] Eye and proboscis of a common fly.

[6] Potato-starch, blanket-hair, portions of feathers.

APPARATUS REQUIRED IN MICROSCOPICAL INVESTIGATION.

I.—The Microscope.

NECESSARY.	ADVANTAGEOUS.
1. *Microscope* with large stage, firm tripod stand, coarse and fine adjustments, double mirror, and arrangement for inclining body; generally termed the *Student's Microscope.*—p. 10. The student's microscope with two powers and bull's-eye condenser costs from five to ten guineas.	*Large Microscope* provided with moveable stage and all the modern improvements. With two powers, this instrument costs from 20 to 30 guineas. *Microscope for Travelling.*—p. 12.
2. *Object glasses.*—1. *The inch* magnifying from 30 *to* 40 *diameters*, the glasses of which can be removed one by one, so that lower powers can be obtained. 2. *The quarter* of an inch magnifying about 200 *diameters.* These glasses should *define well*, the field should be *perfectly flat* and free from *coloured fringes*, and they should admit a sufficient amount of light.—p. 6.	3. *Two-inch object glass.* 4. *Eighth of an inch.*

II.—Accessory Apparatus.

3. *Diaphragm plate.*—p. 17. 4. *Bull's-eye condenser.*—p. 14.	*Gillett's achromatic condenser.*—p. 18. *Polariscope.*—p. 18. *Spot glass.*—p. 16.

For Artificial Illumination.

NECESSARY.	ADVANTAGEOUS.
5. Small French moderator lamp.—p. 19.	Smith & Beck's camphine lamp or Mr. Highley's gas lamp.—p. 19.

III.—Apparatus for Drawing Objects.

6. *Neutral tint glass reflector.*—p. 20.
7. Common hard pencils, steel pens, Indian ink, fine Bristol board.

IV.—Apparatus for Measuring Objects and for Ascertaining the Magnifying Power of the Object Glasses.—p. 21.

8. *Stage micrometers* divided into 100ths and 1000ths of an English inch.—p. 22. *Neutral tint glass reflector.*	Nobert's lines, which may be used also as *test objects.*—p. 22.

V.—Instruments and Apparatus for General Purposes.

9. *Wire retort stand.*—p. 25.	*Water bath.*—p. 26.
10. *Tripod wire stands.*—p. 25.	
11. *Spirit lamp.*—p. 25.	
12. *Evaporating basins.*	
13. *Watch glasses.*—p. 29.	
14. *Thin glass.*—p. 29.	
15. *Plate-glass slides.*—p. 29.	

VI.—Instruments for Making Dissections and for Cutting Thin Sections of Soft Tissues.

16. *Common scalpels.*—p. 26.	*Valentin's knife.*—p. 27.
17. *Double-edged scalpel.*—p. 26.	

NECESSARY.	ADVANTAGEOUS.
18. *Scissars.*—Ordinary form and two small pair, one with curved blades.—p. 27.	*Spring scissars.*—p. 28.
19. *Needles* mounted in handles.—p. 28.	*Compressorium.*—p. 56.
20. *Needles flattened* near the points.	
21. *Forceps.*—One pair of ordinary dissecting forceps, and one pair with curved blades.—p. 28.	

For Dissecting under Water.

22. *Glass dishes* of various sizes from an inch to two inches in depth.—p. 53.	*Large bull's-eye condenser*, for condensing a strong light upon the object.—p. 53.
23. *Loaded corks.*—p. 53.	
24. *Fine pins* and *thin silver wire.*	
25. *Tablets* of wax and gutta percha.—p. 53.	

For Cutting Thin Sections of Hard Tissues.

26. *Saw* with fine teeth, for cutting thin sections of bone.	*Section cutter* for cutting thin sections of wood.—p. 56.
27. *Hones* for grinding the sections thinner and polishing them.—p. 55.	
28. *Strong knife* for cutting thin sections of horn, &c.—p. 55.	

VII.—Cements.

29. *Brunswick black*, containing a few drops of a solution of India rubber in coal naphtha.—p. 30.	*Gold size.*—p. 30.
30. *Marine glue.*—p. 31.	*Solution of shell-lac*—p. 30.
31. *Gum water.*—p. 32.	
32. *Gum* thickened with *starch* or *whiting.*—p. 32.	
33. *French cement* composed of lime and India rubber.—p. 33.	

VIII.—Preservative Fluids.

NECESSARY.	ADVANTAGEOUS.
34. *Spirit and water.*—p. 34.	*Gelatine & glycerine.* —p. 38.
35. *Glycerine.*—p. 35.	
36. *Solution of naphtha and creosote.*—p. 36.	
37. *Chromic acid.*—p. 37.	
38. *Turpentine.*	
39. *Canada balsam.*—p. 32.	

IX.—Apparatus Required for Making Cells and for Cutting and Grinding Glass.

40. *Brass plate* for heating slides to which marine glue is to be applied.—p. 26. *Cements* before enumerated.—p. 30.	*Shadbolt's apparatus.* —p. 41.
41. *Small brush* made of bristles.	
42. *Tinfoil* of different degrees of thickness.—p. 41.	
43. *Writing diamond.*—p. 41.	*Brass rings* for cutting circles of thin glass.—p. 41.
44. *Glazier's diamond.*—p. 41.	
45. *Flat stone* or *pewter plate* for grinding glass.—p. 41.	*Wooden forceps* for holding glass slides. —p. 28.
46. *Emery powder.*	
47. *Old knife* and small chisel for cleaning off superfluous glue.—p. 42.	
48. *Solution of potash* (liquor potassæ).	
49. *Sections of glass tubes* and of thick square vessels, of various sizes, for making cells for the preservation of injections.—p. 44.	*Shallow concave glass cells.* *Moulded glass cells.*—p. 45.

X.—Apparatus for Preserving Objects in Air, Fluid, and Canada Balsam.

50. *Cells* of various sizes, before enumerated.—p. 44. *Brunswick Black.* Gum thickened with whiting.—p. 32.	*Apparatus* for pressing down the thin glass cover while the cement is drying.
51. *Thin glass* cut of the requisite size. *Preservative solutions.*—§ VIII., p. 34.	

NECESSARY.	ADVANTAGEOUS.
52. *Watch glasses* to soak sections in the preservative fluids.—p. 29.	*Bell jar* with vessel for *sulphuric acid*, covered with wire gauze.
53. *Glass shades* to protect recently mounted preparations from dust. —note, p. 63.	
54. *Brass plate.*—p. 26. *Canada balsam.*—p. 32. *Needles* to remove air bubbles.	*Air-pump* to remove air bubbles from the interstices of a tissue.—p. 66.

XI.—Apparatus Required for the Separation of Deposits from Fluids and for their Preservation.

55. *Conical glasses.*—p. 68.	*Glass troughs for Zoophytes.*—p. 46.
56. *Pipettes.*—p. 69.	
57. *Wash-bottle.*—p. 71.	
58. *Cells for the examination of infusoria.*—p. 70.	
59. *Animalcule cage.*—p. 46.	

XII.—Instruments and Apparatus Required for Making Injections.

60. *Injecting syringe*, holding from half-an ounce to an ounce.—p. 73.	
61. *Pipes* of various sizes.—p. 74.	*Stop-cocks.*—p. 74.
62. *Corks* for stopping the pipes.—p. 74.	
63. *Needle* for passing the thread round the vessel.—p. 74.	
64. Thread of different degrees of thickness.	
64*Bull's-nose forceps for stopping vessels.—p. 74.	

For Making Opaque Injections.—p. 74.

65. *Size* or *gelatine.*—p. 74.	Injecting can, made of copper.—p. 74.
66. *Vermilion.*—p. 74.	
67 *Bichromate of potash* and *acetate of lead* for making solutions for precipitating yellow *chromate of lead.* —p. 75.	

NECESSARY.	ADVANTAGEOUS.
68. *Carbonate of soda* and *acetate of lead* for making solutions for precipitating *carbonate of lead*.—p. 75.	

For Making Transparent Injections.—p. 76.

69. *Ferrocyanide of potassium.* "*Muriated tincture of iron.*" *Glycerine* and *Spirits of wine* for preparing the *Prussian blue injecting fluid.* p. 78.	*Carmine.*—p. 77.

XIII.—Chemical Analysis in Microscopical Investigation.

70. *Platinum foil.*	*Small platinum capsule.*
71. *Test tubes and rack.*	
72. *Small tubes* about an inch or an inch and a half in length.	*Small flasks.*
	Platinum wire.
73. *Stirring rods.*	
74. *Evaporating basins.*	
75. *Watch glasses.*	
76. *Small glass bottles with capillary orifices.*	
77. *Wire triangles, tripods.*	
Small retort stand.	
Reagents.—p. 89.	
78. *Ether.*	
79. *Nitric acid.*	
80. *Acetic acid.*	
81. *Ammonia.*	
82. *Solution of potash.*	
83. *Solution of soda.*	
84. *Nitrate of silver.*	
85. *Nitrate of Barytes.*	
86. *Oxalate of ammonia.*	
87. *Iodine solution.*	
88. *Test Papers.*	

XIV.—Cabinet for Preserving Microscopical Specimens.

Drawers arranged so that the specimens may lie *perfectly flat.*—p. 72.	

INDEX.

Acid, acetic, 89.
—— action of, upon animal structures, 86.
—— in examination of the spinal cord, 88.
—— chromic, 87.
—— nitric, 87, 89.
Acids, effects of, upon animal structures, 86.
Achromatic condenser, 17.
Adipose tissue, 48.
Adjustment screws, 8.
Air-bubbles, 68.
Air, removal of, from the interstices of a tissue, 66.
Alcohol, 34.
—— effects of, in hardening tissues, 87.
Alkalies, effects of, upon animal structures, 86.
Ammonia, 90.
Ammoniaco-magnesian phosphate, 91.
Analyzer, 18.
Angle of aperture, 6.
Animalcule cage, 46.
Apparatus for cutting thin sections of wood, 56.
—— necessary for microscopical research, 11, 25, 112.
Artificial injections, 73.
Balsam, Canada, 32.
Barytes, nitrate, 90.
Binocular microscope, 11.
Blanket hair, 102.
Blue injections, Prussian blue 78.
Boiling tissues, 87.
Bone, examination of, 67.
Brass plate, 26.
Bread crumbs, 102.
Brunswick black, 30.
Bryson, microscope-maker, 9.
Built glass cells, 44.
Bull's-eye condenser, 14.
Burnett's solution, 38.
Cabinets for microscopic preparations, 72.
Camera lucida, 20.
—— photographic, 95.
Canada balsam, 32.
—— mounting preparations in, 65.
Canaliculi of bone, 67.
Cans for injecting, 74.
Capillary vessels, 49.
Carbonate, testing for, 91.
—— of lead, 75.
—— of lime by reflected light, 58.
—— in different media, 58.
Caution necessary in drawing inferences from microscopical examination, 98.
Cells for preserving preparations 40.

Cells for examination of deposits, 46, 70.
—— Brunswick black, 41.
—— gutta percha, 46.
—— tinfoil, 40
—— very thin glass, 29.
Cements, 30.
—— for attaching gutta percha to the glass slides, 31.
—— for attaching cover to large glass cells, 33.
Chemical analysis in microscopical investigation, 85.
—— apparatus required for, 117.
—— reagents, use of, in elucidating structures, 86.
—— tests, application of, to objects under the microscope, 90.
Chevalier, microscope-maker, 9.
Chromic acid, 37, 89.
Chromate of lead, 75.
Chromatic aberration, 6.
Ciliary motion in the kidney of the frog and newt, 52.
Ciliated epithelium, 52.
Clarke, Mr. J. L., method of examining spinal cord, 88.
Cleanliness, importance of, in microscopical investigation, 99.
Cobweb micrometer, 21.
Colouring matters used for injection, 74.
—— Chromate of lead, 75.
—— Prussian blue, 77.
—— vermilion, 75.
Compressorium, 57.
Concave glass cells, 44.
Condenser, achromatic, 17.
—— bull's-eye. 14.
—— Gillett's, 18.
Conical glasses, 68.
Corks for dissection under water, 53.
—— for injecting pipes, 74.
Cotton fibres, 102.
Cover, application of, to cell, 63.
Creosote solution, 36.
Cutting glass, 41.
—— circular pieces of thin glass, 41.
Cutting thin sections of soft tissues, 54.
—— hard tissues, 55.
Dancer, microscope-maker, 9.
Dark-ground illumination, 15
Deane, Mr., his preservative gelatine, 37.
Deep glass cells, 44.
—— made with blowpipe, 45.
Delves, Mr., on photographs, 96.
Dennis, Mr., on glass cells, 44.
Deposits, collecting small quantities of, 69.
Diamond, cutting, 41.
—— writing, 41.
Diaphragm, 9.
Diatomaceæ, siliceous skeletons of, 71.
Dissecting microscopes, 12.
Dissection under the surface of fluid, 52.
Drawing objects, 20.
Drying tissues for microscopical examination, 54, 66.
Epithelium, ciliated, 52.
Ether, 89.
Evaporation and drying, 26.
Examination of deposits from fluid, 68.
—— of objects by reflected light, 14.
—— of objects by transmitted light, 17.
—— of organs in lower animals, 50.
—— of soft tissues, 62.
—— of hard tissues, 55.
—— of substances in different media, 58.
—— of tissues, 49.
—— of muscle, 62.
—— of tissues by boiling previously, 55.
—— of tissues by drying, 54.
—— of tissues, magnifying powers required in, 10.
Extraneous substances, 102.
Eye-pieces, 5.
Eye-piece micrometer, 23.

Fallacies to be guarded against, 98.
Fibres of deal wood, 102.
—— and membranes produced artificially, 101.
Fibrillæ of muscle, 49.
Fish, injecting, 79.
Fixing cells to glass slides, 42.
—— cover on large glass cells, 33.
Flax, 102.
Flesh, examination of, 47.
Fluid for injecting, preparation of, 78.
Focus, arrangement for altering, 8.
Forceps, 28.
—— for stopping vessels in injecting, 74.
Frauenhofer, microscope-maker, 9.
Frere, Dr., his method of making thin glass cells, 43.
French cement, composed of lime and India-rubber, 33.
Frog, injection of, 81.
Gas lamp, 19.
Gelatine, preservative, 37.
—— and size for injecting, 74.
Generalization in microscopical inquiries, 98.
Gillett's condenser, 18.
Glands, injection of, 82.
Glass cells, 43.
—— for polishing thin sections of bone, &c., 55.
Glasses for dissecting under water, 53.
Globule, 93.
Glycerine, 35.
Goadby, Dr., preservative solution, 38.
Goniometer, 23.
Granule, 93.
Grinding glass, 41.
—— thin sections of hard tissues, 55.
Gum, 32.
Guy, Dr., his cells, 46.
Hair, transverse and longitudinal sections of, 55.
Hardening tissues, solutions for, 87.
Herepath, Dr., on crystals of iodo-quinine, 18.
Highley, Mr., gas lamp, 19.
Hones for grinding hard tissues, 55.
Huyghenian eye-piece, 5.
Igniting substances to remove organic matter, 71.
Illumination, dark ground, 16.
—— of opaque objects, 15.
—— of transparent objects, 17.
Inclining microscope, arrangement for, 8.
Influence of media upon the microscopical appearance of substances, 57.
Infusoria, examination of, 70.
Injecting, 72.
—— cans, 74.
—— Coloring matters employed in, 75.
—— fishes, insects, mollusca, 79.
—— operation of, 80.
—— pipes, 74.
—— syringes, 73.
—— Prussian blue fluid for, 78.
Injections mounted in Canada balsam, 84.
—— of liver, 83.
Instruments, 26.
Jackson, Mr., his micrometer, 22.
Kidney of newt, 51.
Knives, dissecting, 26.
—— thin-bladed, 26.
—— Valentin's, 27.
Lacunæ of bone, 67.
Ladd, microscope-maker, 9.
Lamp, Highley's gas microscope, 19.
—— camphine, Smith and Beck's 19.
—— spirit, 25.
Lieberkuhns, 15
Lime, carbonate of, 53.

Lime, and India rubber cement, 33.
—— phosphate of, 91.
Loaded corks, 53.
Lymphatic vessels, 48.
—— feathers mistaken for, 102.
Magnifying power, method of ascertaining, 24.
Marine glue, 31.
Matthews, Mr., new form of Valentin's knife, 27.
Matters, extraneous, 101.
Measuring, method of, 21.
—— objects with the camera, &c., 23.
Mercurial injection, syringe for, 79.
Merz, microscope-maker, 9.
Methylated spirits of wine, 35.
Method of examining soft tissues, 49.
Micrometer, 21.
Microscope, 4.
Microscope, arrangement of, for drawing, 20.
—— binocular, 11.
—— choice of a, 10.
—— essential points in construction of, 10.
—— makers of, 9.
—— simple and compound, 4.
—— students', 10.
Microscopical investigation, chemical analysis in, 85.
Micrometer, cobweb, 21.
—— eye-piece, 23.
—— stage, 22.
Mirror, 7.
Molecules, 93.
Mollusca, injecting, 79.
Mounting objects in Canada balsam, 65.
—— in the dry way, 60.
—— in fluid, 61.
—— preparations in large glass cells, 33,
—— sections of bone, 67.
Muscular fibre, 48.
Nachet, microscope-maker, 9.
Naming preparations, 72.
Naphtha and creosote solution, 36.
Natural injections, 73.
Needles, 28.
—— for passing thread round vessels, 74.
Negative eye-piece, 5.
Neutral tint-glass reflector, 20.
Newt, kidney of, 51.
Nicol's prism, 18.
Nitrate of barytes, 90.
—— of silver, 90.
Nitric acid, 90.
—— effects of, upon animal structures, 87.
Nobert's micrometer lines, 22.
Notes of observations, 92.
Oberhäuser, microscope-maker, 9.
Object-glass, 6.
Oil-globules, 68.
Operation of injecting, 80.
Parabolic reflector, 16.
Pewter plate for grinding glass, 42.
Phosphates of lime, 91.
—— triple or amoniaco-magnesian, 91.
Photography, application of, to microscope, 95.
Pillischer, microscope-maker, 9.
Pins for dissecting, 28.
Pipe, injecting, 74.
Pipettes, 69.
Pistor, microscope-maker, 9.
Plate glass slides, 29.
Ploesl, microscope-maker, 9.
Polarized light, 18.
Polishing thin sections of hard tissues, 55.
Positive eye-piece, 5.
Potash, 9.
Powell and Lealand, microscope-makers, 9.
Preparations mounted in the dry way, 60.
—— aqueous fluids, 61.
—— Canada balsam, 65.

Preparing objects for examination, 52.
Preservation of objects in aqueous fluids, 61.
Preservative fluids, 34.
—— Gannal's solution, 39.
—— gelatine and glycerine, 38.
—— glycerine, 35.
—— Goadby's solution, 38.
—— other saline solutions, 39.
—— solution of chromic acid, 37.
—— solution of naphtha and creosote, 36.
—— spirit and water, 34.
—— Thwaites' fluid, 36.
Price's patent glycerine, 35.
Prussian blue, 77.
Quekett, Mr., his arrangement for condenser, 17.
Reagents, 89.
—— application of, to minute quantities of matter, 90.
—— effects of, upon animal structures, 86.
—— kept in small glass bulbs with capillary orifices, 90.
Red injections, 75.
Reflected light, 14.
Retort stand, 24.
Richardson, Dr., method of preserving tissues in nitrogen, 39.
Rings, brass, for cutting circular pieces of thin glass, 41.
Ross, microscope-maker, 9.
Rotifers, examination of, 70.
Saline preservative solutions, 39.
Salmon, Mr., microscope-maker, 9.
Scalpels, 26.
Scalpels, double-edged, for cutting thin sections, 26.
Schick, microscope-maker, 9.
Schmidt's goniometer, 23.
Scissars, 27.
—— for cutting thin sections, 27.
Sealing-wax varnish, 30.
Sections of hair, longitudinal and transverse, 55.
Sections of hard tissues, 55.
—— of soft tissues, cutting, 54.
Shadbolt, Mr., apparatus for making cells, 41.
Shell-lac, solution of, 30.
Siliceous skeletons of diatomaceæ, 71.
Silver, nitrate of, 90.
Size and gelatine for injecting, 74.
—— gold, 30.
Slides, 29.
Smith and Beck, microscope-makers, 9.
—— lamp, 19.
Soda, caustic, 89.
—— and alcohol, for hardening tissues and making them transparent, 88.
Soft tissues, cutting thin sections of, 54.
Spherical aberration, 6.
Spot-glass, 16.
Spinal cord, method of obtaining sections of, 88.
Spirit-lamp, 25.
Spirits of wine, methylated, 34.
Stage micrometer, 22.
—— of microscope, 8.
Starch-globules, 102.
Steam bath, 26.
Steel mirror, 20.
Stones for grinding thin sections of hard tissues, 55.
Stopcocks, 74.
Stops, 15.
Syringe for mercurial injections, 79.
—— injecting, 73.
Tables for practising manipulation, 104.
Tea-leaves, 102.
Teeth, sections of, 56.
Testing for chlorides, phosphates, sulphates, carbonates, lime, magnesia, 91.
Test objects, 22.
—— tubes, 71.
Tests kept in bottles, or in small bulbs, 90.

Tests method of applying to microscopieal examination, 86.
Thin glass, 29.
—— cells, 43.
—— eover, plaeing it upon the cell, 63.
Thwaites' fluid, 36.
Tissues, the demonstration of, 49.
—— whieh may be mounted in air, 60.
—— whieh may be mounted in fluid, 61.
—— which may be mounted in Canada balsam, 65.
Traeheæ, 104.
Transmitted light, 17.
Transpareney of objects too great, 100.
Transparent objeets, illumination of, 17.
Travelling microscopes, 12.
Triple phosphate, 91.
Tripods, 25.
Triton, kidney of, 51.
Valentin's knife, new form of, 27.
Vallisneria, examination of, 65.
Varnish, sealing-wax, 30.
Vermilion, 75.
Vessels, examination of, 72.
Warington, Mr., his mieroscope, 12.
Wash-bottle, 71.
Wateh-glasses, 71.
Water-bath, 26.
Wax and gutta pereha, tablets of, 53.
White, Mr., his arrangement for fixing on the thin glass cover, 64.
White injeetions, 75.
—— light for illumination, 19.
Wood, seetions of, 56.
Wooden foreeps, 28.
Yellow injeetions, 75.
Zoophytes, examination of, 70.

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