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Memoir on Inventing the Confocal Scanning Microscope

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This is what I remember about inventing the confocal scanning microscope in 1955. It happened while I was making a transition between two other theoretical preoccupations and I have never thought back to that period until Alan Boyde suggested writing this memoir. When I read the following account, the plot seems more coherent now than it ever did in those times of the past. Perhaps, though, those activities which seemed to me the most spontaneous were actually those which unconsciously were managed the most methodically.

The story actually begins in childhood, for my father was an ophthalmologist and our home was simply *full* of lenses, prisms, and diaphragms. I took all his instruments apart, and he quietly put them together again. Later, when I was an undergraduate at Harvard in the class of 1950, there were new wonders every day. I studied mathematics with Andrew Gleason, neurophysiology with John Welsh, neuroanatomy with Marcus Singer, psychology with George Miller, and classical mechanics with Herbert Goldstein. But perhaps the most amazing experience of all was in a laboratory course wherein a student had to reproduce great physics experiments of the past. To ink a zone plate onto glass and see it focus on a screen; to watch a central fringe emerge as the lengths of two paths become the same; to measure those lengths to the millionth part with nothing but mirrors and beams of light—I had never seen any things so strange.

For graduate studies I moved to Princeton to study more mathematics and biology, and wrote a theoretical thesis on connectionistic learning machines—that is, on networks of devices based on what little was known about nerve cells. I studied everything available about the physiology, anatomy, and embryology of the nervous system. But there simply were too many gaps; nothing was known about how brains learn. Nevertheless, it occurred to me, you might be able to figure that out—if only you knew how those brain cells were connected to each other. Then you could attempt some of what is now called "reverse engineering"—to guess what those circuits' components do from knowing both what the circuits do and how their parts are connected. But I was horrified to learn that even those connection schemes had never been properly mapped at all. To be sure, a good deal was known about the *shapes* of certain types of nerve cells, because of the miraculous way in which the Golgi treatment tends to pick out a few neurons and then stain all the fibres that extend from them. But this permits you to visualize only one cell at a time, whereas to obtain the required wiring diagram you need to make visible *all* the cells in a three dimensional region. And here was a critical obstacle: the tissue of the central nervous system is solidly packed with interwoven parts of cells. Consequently, if you succeed in staining all of them, you simply can't see anything. This is not merely a problem of opacity because, if you put enough light in, some will come out. The serious problem is scattering. Unless you can confine each view to a thin enough plane, nothing comes out but a meaningless blur. Too little signal compared to the noise: the problem kept frustrating me.

After completing that doctoral thesis, I had the great fortune to be invited to become a Junior Fellow at Harvard. That three-year membership in the Harvard Society of Fellows carries unique privileges; there is no obligation to have students, responsibilities, or supervisors, and all doors to the university are opened; one is bound only by a simple oath to seek whatever seems the truth. This freedom was just what I needed then because I was making a change in course. With the instruments of the time so weak, there seemed little chance to understand brains, at least at the microscopic level. So, during those years I began to imagine another approach. Perhaps we could work the other way; begin with the large-scale things minds do and try to break *those* processes down into smaller and smaller ingredi-

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ents. Perhaps such studies could help us to guess more about the low-level processes that might be found in brains. Then, perhaps we could combine what we learned from both "top down" and "bottom up" points of view—and eventually close in on the problem from two directions.

In the course of time, that new top down approach did indeed become productive; it soon assumed the fanciful name, Artificial Intelligence (AI). But that is a different story, and the only part that is relevant here was what happened to me in that interlude. I now felt that while it might take decades to learn enough more about the brain, AI could be tackled straight away—but my ideas about doing this were not yet quite mature enough. So (it seems to me in retrospect) while those ideas were incubating I had to keep my hands busy and solving that problem of scattered light became my conscious obsession. Edward Purcell, a Senior Fellow of the Society of Fellows, obtained for me a workroom in the Lyman Laboratory of Physics, with a window facing Harvard Yard and permission to use whatever shops and equipment I might need. (That room had once been Theodore Lyman's office. Under an old sheet of shelf paper I found a bit of diffraction grating that had likely been ruled, I was awed to think, by the master spectroscopist himself.) One day it occurred to me that the way to avoid all that scattered light was to never allow any unnecessary light to enter in the first place. An ideal microscope would examine each point of the specimen and measure the amount of light scattered or absorbed by that point. But if we try to make many such measurements at the same time then every focal image point will be clouded by aberrant rays of scattered light deflected by or from points of the specimen that are not the point you're looking at. Most of those extra rays would be gone if we could illuminate only one specimen point at a time. There is no way to eliminate every possible such ray, because of multiple scattering, but it is easy to remove all rays not initially aimed at the focal point; just use a second microscope (instead of a condenser lens) to image a pinhole aperture on a single point of the specimen. This reduces the amount of light in the specimen by orders of magnitude without reducing the focal brightness at all. Still, some of the initially focused light will be scattered by out-of-focus specimen points onto other points in the image plane. But we can reject those rays, as well, by placing a second pinhole aperture in the image plane that lies beyond the exit side of the objective lens. We end up with an elegant, symmetrical geometry: a pinhole and an objective lens on each side of the specimen. (We could also employ a reflected light scheme by placing a single lens and pinhole on only one side of the specimen—and using a half-silvered mirror to separate the entering and exiting rays.) This brings an extra premium because the diffraction patterns of both pinhole apertures are multiplied coherently: the central peak is sharpened and the resolution is increased. (One can think of the lenses on both sides of the microscope combining, in effect, to form a single, larger lens, thus increasing the difference in light path lengths for point-pairs in the object plane.)

The price of single-point illumination is being able to measure only one point at a time. This is why a confocal microscope must scan the specimen point by point and that can take a long time because we must add all the time intervals it takes to collect enough light to measure each image point. That amount of time could be reduced by using a brighter light—but there were no lasers in those days. I began by using a carbon arc, the brightest source available. Maintaining this was such a chore that I had to replace it by a second best source: zirconium arcs, though less intense, were a great deal more dependable. The output was measured with a low noise photomultiplier which Francis Pipkin helped me design. Finally, the image was reconstructed on the screen of a military surplus long persistence radar scope. The image remained visible for about ten seconds, which was also how long it took to make each scan.

The most serious design problem was choosing between moving the specimen or moving the beam. At first it seemed more elegant to deflect a weightless beam of light than to move a massive specimen. But daunted by the problem of maintaining the three-dimensional alignment of two tiny moving apertures, I decided that it would be easier to keep the optics fixed and move the stage. I also was reluctant to use the single-lens reflected light scheme because of wanting to "see" the image right away! (Not only would dark field be inherently dimmer, but there would also be the fourfold brightness loss that beam splitters always bring.) A more patient scientist would have accepted longer exposure times and assembled the pictures as photographs—which would have produced permanent records rather than transient subjective impressions. In retrospect it occurs to me that this concern for real-time speed may have been what delayed the use of this scheme for almost thirty years. I demonstrated the confocal microscope to many visitors, but they never seemed very much impressed with what they saw on that radar screen. Only later did I realize that it is not enough for an instrument merely to *have* a high resolving power; one must also make the image *look* sharp. Perhaps the human brain requires a certain degree of foveal compression in order to engage its foremost visual abilities. In any case, I should have used film—or at least have installed a smaller screen!

Once I decided to move the stage, this was not hard to accomplish. The specimen was mounted between two cover slips and attached to a flexible platform that was supported by two strips of spring metal. A simple magnetic solenoid flexed the platform vertically with a 60 hertz sinusoidal waveform, while a similar device deflected the platform horizontally with a much slower,

sawtooth waveform. The same electric signals (with some blanking and some corrections in phase) also scanned the image onto the screen. Thus the stage-moving system was little more complex than an orthogonal pair of tuning forks. The optical system was not hard to align and proved able to resolve points closer than a micrometer apart, using 45x objectives in air. I never got around to using oil immersion for fear that it would restrict the depth to which different focal planes could be examined, and because the viscosity might constrain the size of scan or tear apart the specimen.

There is also a theoretical advantage to moving the stage rather than the beam: the lenses of such a system need to be corrected only for the family of rays that intersect the optical axis at a single focal point. In principle, that could lead to better lens designs because such systems need no corrections at all for lateral aberrations. In practice, however, for visible light, opticians can already make wide field lenses that approach theoretical perfection. (This was another thing about optics I had always found astonishing: the mathematical way in which the radial symmetry of a lens causes odd order terms of series expansions to cancel out, so that you can obtain sixth order accuracy by making only two kinds of corrections, of second and fourth order. It almost seems too good to be true that such simple combinations of spherical surfaces—the very shapes that are the easiest to fabricate—can transform entire four dimensional families of rays in such orderly ways.) However, the advantages of combining stage scanning with paraxial optics could still turn out to be indispensable, for example, for microscopes in the X-ray domain for which refractive lenses and half-silvered mirrors may never turn out to be feasible.

In constructing the actual prototype, the electronic aspects seemed easy enough because, a few years earlier, I had already built a learning machine (to simulate those neuronal nets)—and that system contained several hundred vacuum tube circuits. But the world of machining was new to me. Constructing an optical instrument was to live in a world where the critical issue of each day was how to clamp some bar of steel to the baseplate of a milling machine, what sort of cutter and speed to use, and how to keep the workpiece cool. I became obsessed with finding ways to reduce the thermal expansion under the wheel of a grinding machine; no matter how flat a surface seemed, I'd find new bumps the following day. (Perhaps I was haunted by Lyman's ghost.) By the time the prototype was complete, I understood how the principles of kinematic design made most of that precision unnecessary. I could have saved months. Still, the machine shop experience was not wasted. A decade later, it helped me to build a singularly versatile robotic arm and hand.

Scanning is far more practical today because we can use computers to transform and enhance the images. In those days computers were just becoming available and my friend Russell Kirsch was already doing some of the first experiments on image analysis. He persuaded me to try some experiments, using the SEAC computer at the Bureau of Standards. However, that early machine's memory was too small for those images, and we did not yet have adequate devices for digitizing the signals. In subsequent years, both Kirsch and I continued to pursue those same ideas—of closing in on the vision problem by combining bottom-up concepts of feature extraction with top-down theories about the syntactic and semantic structures of images. Eventually, Kirsch applied those techniques to "parsing" pictures of actual cells, while I pursued the subject of making computers recognize more commonplace sorts of things. I should mention that I was also working with George Field (who also helped with the microscope design) on how to use computers to enhance astronomical images. Such schemes later became practical but at that time they, too, were defeated by the cost of memory. I returned to physical optics only once more, in the middle 1960s, in building computer controlled scanners for our mechanical robotics project and in studying the feasibility of using somewhat similar systems in conjunction with radiation therapy.

I also pursued another dream—of a microscope, not optical, but entirely mechanical. Perhaps there were structures that could not be seen—because they could not be selectively stained. What, for example, served to hold the nucleus away from the walls of a cell? Perhaps there was a scaffolding of invisible fibres that one might recognize by plucking them—and then measure the strain, or see other things move. I examined the various micromanipulators that already existed but, finding none that seemed suitable, I designed one which I hoped to use in conjunction with my new microscope. Again, the Society of Fellows came to my aid, this time in the person of Carroll Williams, who invited me to build it in his laboratory. The new micromanipulator was extremely simple: I mounted the voice coils of three loudspeakers at right angles and connected them with stiff wires to a diagonally mounted needle probe. The needle could be moved in any spatial direction, simply by changing the current in the three coils. The only hard part was replacing the coil suspensions with materials free from mechanical hysteresis. The resulting probe could be swiftly moved with precision better than 100 nanometers, over a range of more than a millimeter. (This sensitivity was at first limited by power supply noise. This was solved by using batteries.) To control the probe, my childhood classmate Ned Feder, who was now also working in Williams' laboratory, constructed a three-dimensional electrical joystick by attaching three conductive sheets to the sides of a tank of salt water. Everyone seemed to like *this* instrument, so we left it around in the laboratory, but it was never actually put to use, and I have no idea what became of it. I had planned to measure the infinitesimal forces by applying

very high frequency vibrations to a microelectrode mounted on the probe and correlating the waveforms against the needle deflections. I never got around to this because, by 1956, AI was already on the march.

This is what I remember now, and it may not all be accurate. I've never had much conscious sense of making careful, long range plans, but have simply worked from day to day without keeping notes or schedules, or writing down the things I did. I never published anything about that earliest learning machine, or about the micromanipulator, or even about that robot arm. In the case of the scanning microscope, it was fortunate that my brother-in-law, Morton Amster, not only liked the instrument but also happened to be a patent attorney. Otherwise I might have never documented it at all. The learning machine and the micromanipulator disappeared long ago but, only today, while writing this, I managed to find the microscope, encrusted with thirty years of rust. I cleaned it up, took this photograph, and started to write an appropriate caption—but then found the right thing in a carbon copy of a letter to Amster dated November 18, 1955.

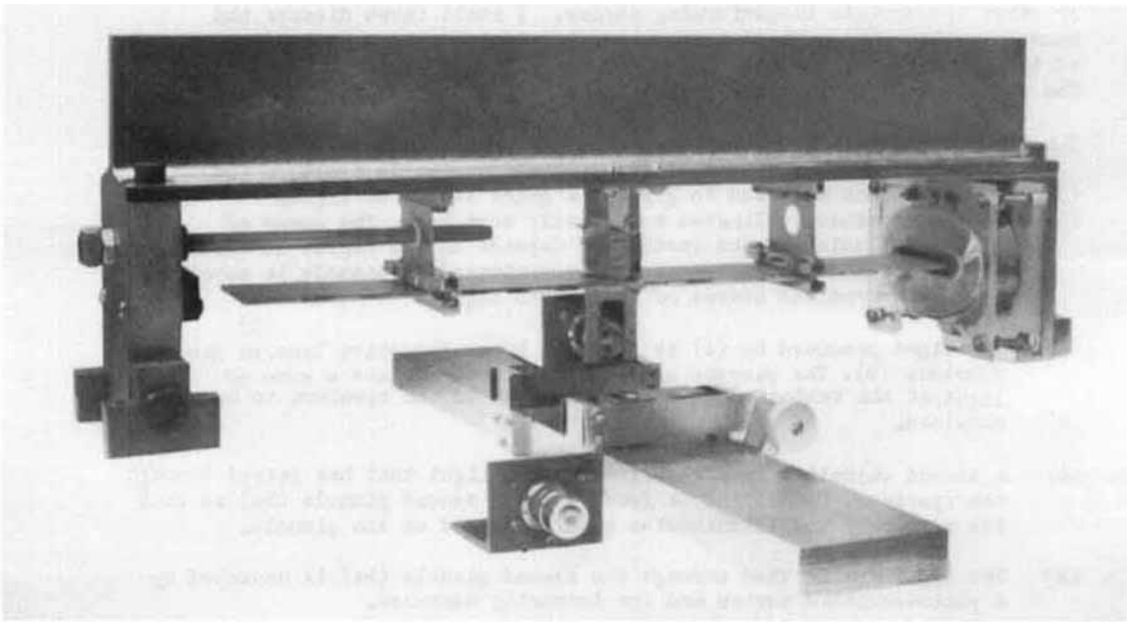


FIG. 1 Photograph of the original confocal microscope dating late 1950's. Photo taken in 1988.

Reproduction of letter dated November 18, 1955:

M.L. Minsky
Double focussing Stage scanning microscope Nov 18 1955 1

To Lorton Amster: Details of principles of operation and application. The following further explains the mode of operation of the instrument which I have built and explained to you in previous letters and conversations.

I will first describe the ~~actu~~ details of the essential optical innovations of the system. It will be recalled that in my instrument an image of a specimen is formed by a point by point examination of the optical properties of the specimen (this point by point examination to be called "scanning") and the information thus obtained is then displayed on an oscilloscope or other appropriate image-forming device. I shall first discuss the manner in which the optical information is obtained at an individual point of the specimen;this is the "double focussing" feature of the instrument. The second basic feature,the "stage scanning" feature will be taken up later.

I.A Let me first describe the essential features of the optical path of the microscope. A source of light(1) and a pinhole aperture (1a)
(1) or equivalent are used to provide a point source of light.
(1a) Any other collimated source will work here. The power of resolution of the instrument depends on the degree to which perfect collimation can be approximated. A pinhole is merely a convenient source of collimated light.

(2) The light produced by (1) is focussed by an objective lens (2) on the specimen (3). The purpose of this lens is to produce a cone of light,at the vertex of which is the point of the specimen to be
(3) examined.

(4) A second objective lens recollimates the light that has passed through the specimen. This light is focussed on a second pinhole (5a) so that the vertex of the illumination cone is imaged on the pinhole.

(5) The light transmitted through the second pinhole (5a) is detected by a photosensitive device and its intensity measured.

~~I.B~~ ~~Before discussing the manner in which this information about the optical properties of a point of the specimen is used to produce an image of the specimen,I will discuss the ways in which this instrument has advantages over conventional optical devices~~

Now the above description is that of the instrument in its simplest form. The light intensity of the light transmitted through each point of the specimen is then recorded and the resulting information is displayed to make a complete image of the specimen. How this may be done is discussed in section II below. Following this,I will discuss how,by inserting into the optical path described in section I above,certain stops,filters,etc., several new techniques of microscopy may be obtained,each of which I believe represent new innovations not to be found within the prior art.

II. In ~~order~~ order to produce a useful image of the specimen,the information obtained by the use of the optical system of section I must be obtained in a like manner for a great many points of the specimen. Points of the specimen can be ~~selec~~ selected in some systematic manner,In particular,it was found convenient to "scan" over the specimen in the manner in which an image is generated on the screen of a television reciever;this method will here be called the "raster" scanning method. In the "stage-scanning" method described herein this scanning is done as follows:

(I wish to remark here that I regard "double focussing" and "stage scanning" as two separate innovations;it would be possible to use each independently of the other in microscopy;the fact is that when both are used we obtain a very convenient and versatile instrument.)

In the"stage-scanning" microscope no part of the optical system need be in motion except the specimen and its immediate mounting. The specimen itself is moved in some regular pattern in such a way that the vertex of the illumination cone described in (3) above describes a raster within the specimen. This raster must of course be fine enough to reveal the details of the specimen which are being studied. The advantage of this method (of moving the specimen rather than parts of the optical system) is that once the optical system has been adjusted for one point of the specimen,no further adjustments will be required,in general, for examination of other parts. In the instrument that I have constructed, the motion of the specimen is obtained by mounting the specimen on an electrically driven tuning fork,which tuning fork in turn is mounted so as to move in a direction perpendicular to the faster vibration of the tuning fork. The result is that the specimen is moved in such a manner that the illumination point described a"raster"within the specimen. Many other ways of moving the specimen are imaginable;the innovation is the very idea of such motion. The tuning fork method just described is particularly convenient in that the the electric signals which determine the position of the specimen can also be conveniently be used to determine a corresponding position for the beam of a cathode ray oscillograph or other two-dimensional display device,and the information obtained by the photocell (5) can be displayed (for example,as brightness)on this two dimensional display,thus forming an image of the specimen.

A feature of this instrument which is not obtainable in conventional microscopy,is that there is no necessity that the plane of the specimen being examined need be perpendicular to the optical axis of the instrument or that of the mounting of the specimen. For the motion of scanning may be made to include a component along the optic axis,(limited only be the working "working distance" of the objectives". This feature may be of great value in microscopy.

(IIa) A converse of this system would be to fix the specimen and move the entire optical system. This would in general be much less convenient,but in the case that the specimen of interest happens to be immovable,it might be useful.

(IIb?) A related method,which I would not consider a converse,would be to move only the two pinholes. As will be explained,however,such an instrument would lose an essential optical aspect of the present invention,in that all light rays of importance are in the planes containing the optic axis.

III. I will now describe certain features of the system described above,and then certain features resulting when some modifications are introduced. Each of these features results in new domains of applicability.

A. The optical system as described has the novel feature that (///):all light rays originate at a point of the optical axis of the instrument,and only rays which terminate at another point of the optic axis (i.e.,at 5a)are accepted by the photosensitive element. Thus only rays of axial origin the optical elements of the system deal only with what may be called "axial cones of light",i.e., families of rays which originate or terminate on a single point of the optical axis. The following are among the very important consequences of this fact

A1: The lenses of the system have only one optical requirement;that of bringing light originating at one point to a focus at another point. ((Note that in ordinary microscopy each lens is required to do this not only for one point,but to do it simultaneously for every individual point of the field of view. This tremendous requirement has never been realized in practice and in conventional lens design it has always been necessary to make a compromise between different aspects of good design,all of which have never been acheived at once.))

Because only a single point on the lens axis need be considered in lens design for this instrument,it follows that an essentially perfect lens for this application need be corrected only for Spherical Abberation and for Longitudinal Chromatic Abberation.(If monochromatic light be used even the latter is unnecessary). Convential microscope lenses need be corrected as well for Coma,Astigmatism,Curvature of field,Distortion of field,Lateral chromatic abberation as well. Thus with the simpler lens design problem,it should be practical to build objectives for this microscope having considerably higher relative apertures,and higher resolving powers,than has been obtainable in conventional microscopes. It should also be practical to make larger lenses with greater working distances more easily. Another problem in conventional systems which is here eliminated is that of obtaining equal relative aperture for each point of the field of view.

A2. Because of the fact that the lenses are not required to form images,it would be relatively easy to make reflection focussing objectives for use with relatively short wave ultraviolet and X-ray illumination. While it is very difficult to form images with these radiations,it would be a much simpler application to produce a single point focus. For this application we need simply replace the objective lens with an elliptiaal(or parabolic) reflecting surface,and supply the radiation from a suitably collimated source,e.g.,a pinhole.

B. Because of the single point field of view (see A and A1 above) this optical system has the novel property that every position along the optical axis is a potential aperture stop location. Now just as the small field of view can greatly simplify lens design problems,this fact about the aperture stops both greatly simplifies most problems in ordinary microscopy and makes possible a number of new microscopic techniques.

B1. In a conventional microscope,in order to obtain a variable aperture stop,it is necessary to use something like an iris diaphragm. Here it is sufficient to move a perforated plate along the optic axis.

B2. Use of an "annular" stop is very popular in microscopy. If different sizes of annular stops are required,it is necessary to obtain a series of different size plates,in ordinary microscopy,and to change the size of the annulus requires interruption of work. With the new instrument, the effective size of an annulus can be continuously varied,without interruption of work,by merely moving a single annulus along the optic axis. If the outer border of this annulus were adjustable,both the diameter and the width of the annular border could be controlled continuously. Equivalently,given only an opaque disc and a perforated plate,of appropriate sized,equipment of negligable expense,effectively annuli,again with both parameters widely variable,can be obtained,by moving the two stops up and down the axis.

B3. When applied to phase contrast and other methods of contrast microscopy,this feature of effectively continuously varying the dimensions of stops has great value, for the conventional forms of many such devices do not lend themselves easily to continuous variation.

B4. Because every point is an aperture stop, many aspects of microscopy that require high precision in the location of stops and other optical parts can be simplified in that high accuracy may be unnecessary.

C. Because of the arrangement of two objective lenses and two pinholes/ apertures, the system is capable of a resolving power somewhat greater than is currently believed possible for a given type of lens. In order to explain this it is necessary to use some mathematical concepts: Suppose that we have a point source of illumination at (1). Then for a given objective lens (2) the specimen will be illuminated by a diffraction pattern in the focal plane of the lens (2). (Although for some purposes it is convenient to assume that only a single point of this plane is illuminated, for the examination of resolving power diffraction effects must be considered.) Now if R denotes the position of a point in this focal plane, let K(R) denote the illumination in this plane. (K(R) is here assumed to be a vector function carrying phase and amplitude.)

Now in the conventional microscope resolving power can be defined as a measure of the dispersion of the distribution K(R) for its objective. For the light that reaches a given point of the image plane of the microscope has an origin that, on the average, is distributed like K(R). But in the double-focussing microscope described herein, the very illumination of the specimen is described by the distribution K(R). Then (by the reciprocity principle of non-optical systems) for any point R, and for the transmission at that point and because of the diffraction of the second objective (4) the light originating at R and entering the second pinhole (5a) is attenuated by the factor K(R) where this time the K(R) is due to the second objective. If we assume that the two objective lenses have the same character, then the illumination distribution as seen from the second pinhole will look like $(K(R))^2$. Now it is a fact that for the usual measures of the dispersion which are interpreted as a measure of resolving power, when applied to a diffraction distribution in which most of the energy is in a smooth central peak, will give a better result for the square of the distribution than for the distribution itself. For a Gaussian diffraction distribution, which is not far from that of an ideal lens (so far as energy distribution is concerned) the improvement in resolution is of the order of 1.41. A comparable improvement has been observed in practice.

NOTE: It would be a serious mistake to identify the conventional microscope condenser with objective and "condenser" as constituting a "double focussing" microscope in the sense as it is used herein. Unless both pinholes are present the remarks in section C above cannot be applied to the microscope. The purpose of the condenser in an ordinary microscope is used, in fact, to obtain adequate and equal illumination at each point, and need not be in good focus for this purpose, in general. In the present instrument, on the other hand, the first objective is used to obtain the greatest possible difference in illumination between points, and it is crucial that it be focussed as precisely as possible. The only point of similarity between the conventional "condenser" and my objective (2) lies in the fact that they both may employ lenses.

D. Light scattered from other than the central focal point is rejected from the system to a novel extent. This results in a reduction of blurring, increase in signal-to-noise ratio, increase in effective resolution, and the possibility of high resolution light microscopy through unusually thick and/or highly scattering specimens. I show below how these results can be acheived.

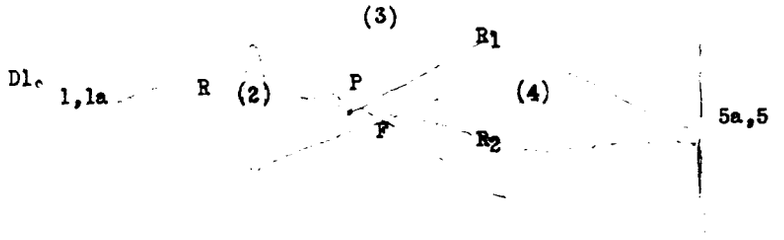


Fig. 1

Consider the path or light rays through the system as shown in Fig.1. The extreme rays illustrate the typical course of unscattered light, eventually entering the pinhole 5a. The ray R, however, is assumed to be scattered at the point P, a point which is not at the location F, the dual focal point of the optical system. The ray R is assumed to split into a number of secondary rays, for example R_1 and R_2. Note that both R_1 and R_2, as well as any other rays that may be scattered from P are eventually rejected from the system, R_1 immediately, and R_2, when it strikes the plate 5_a without passing through the pinhole. The only way in which such scattered light can reenter the system is to be scattered again. For R_2 to reenter, it would have to be deflected again, and this deflection would be constrained to just one direction, i.e., co-linear with the focal point F. This second order scattering in just the right direction has very low probability compared with the first order scattering if the specimen is not so dense as to transmit almost no direct light. For the ray R_1 or others in its class, third order scattering would be required to return it to the photocell, hence its rejection is even more effective.

The consequence of this rejection of light scattered from points other than F is that in media in which scattering makes ordinary microscopy (including the various forms of high contrast microscopy) impractical, this system will often work effectively.

IT should be noted that the rejection of light scattered from points other than F does not imply the rejection of light scattered at F. Ordinary dark-field methods may be used by selection of proper aperture devices just as in ordinary microscopy.

D2 By using the system with the pinholes slightly out of line, it is possible to obtain darkfield effects where the illumination originates (by scattering) within the specimen, and can be observed with the full aperture of the second microscope. Use of slits, very close pairs of pinholes, and annular pinholes in combination with color filters and phase retardation plates yield so large a variety of different contrast modes of operation.

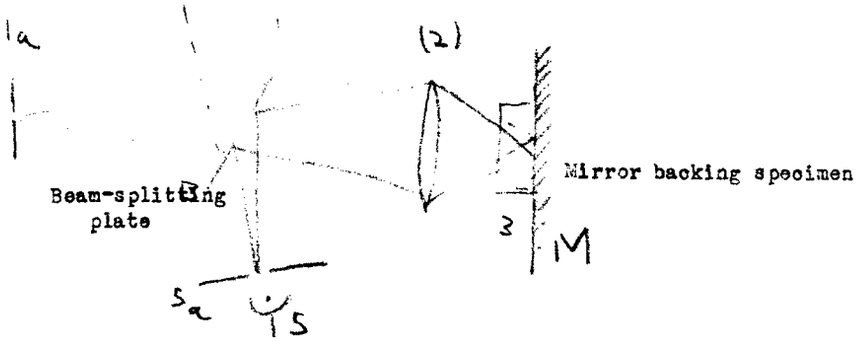
D3.

E. The system can be varied in a number of ways. A number of these described:

E1. If objective (4) is replaced by a spherical mirror focussing the light back on (3), and a beam splitting plate placed between (1) and (1a), OR a beam splitting plate between (1a) and (2) and a second pinhole supplied, then we obtain a system which is similar except that light passes twice through the specimen, and little apparatus is required one one side of the specimen. Alignment problems would not necessarily be simplified by this change.

E2. The following modification is of some interest. The specimen is mounted on the reflecting surface of a plane mirror. The objective (4) is eliminated and a beam splitting plate is introduced between (1a) and (2). The pinhole (5a) and photocell (5) are located so as to receive the reflected light. A special objective would be required here, since (2) has to focus at two different working distances. In view of the simplifications of lens design mentioned above in A1, this should be possible.

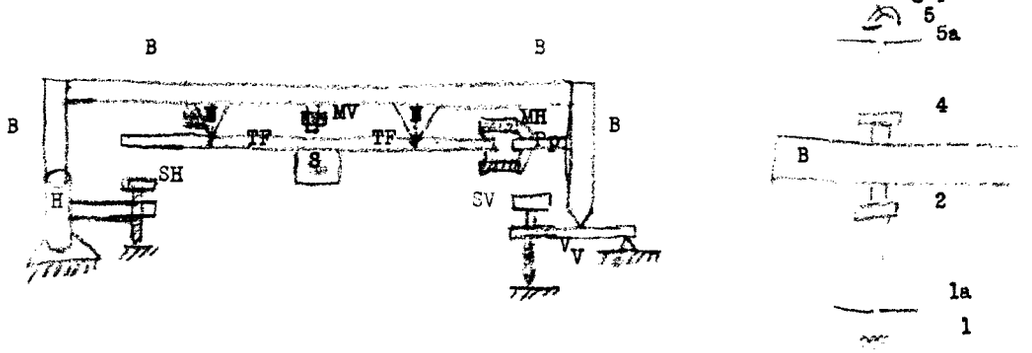
Minsky: Double-focussing ~~beam-split~~ stage-scanning microscope 6



Although this instrument requires a more highly corrected objective, it needs only one such.

F. Details of the stage scanning device: Below is illustrated the method of stage-scanning that I use in practice.

The specimen (3) S is mounted on a tuning fork TF(vibrating plate) which is suspended at its nodes N,N from the main frame or "bridge"B. The vibrating plate is excited at its natural frequency by the electromagnet MV, causing the specimen to vibrate in the vertical direction. The entire vibrating plate



is moved horizontally by the magnet (solenoid) MH, whose action is enhanced by the pole piece Pp. This horizontal motion is damped by the action of viscous damping material placed against the nodal suspension N. These two magnets produce an adequate scanning pattern. The coarse adjustment screws SH and SV act through corresponding linkages H and V to effect coarse positioning of the specimen, or, if desired, manual ~~positioning of the~~ control over scanning. The diagram at the right is a top view showing position of the optical system relative to the bridge B.

G. Ordinary direct eye viewing of the specimen simultaneous with scanning can be obtained by combining (1) a beam splitting plate or prism at the viewing end with appropriate ocular lens and (2) stroboscopic illumination of the whole specimen through beam splitter at illumination end, with (3) the stroboscopic illumination synchronized with the fast component of the scanning pattern, e.g., with the period of the vibrating plate TF in D above. The output of the photocell (5) in response to the stroboscopic illumination can be removed electronically or allowed to remain as a bright or dark line across the image as a reference for the phase of the vibration.

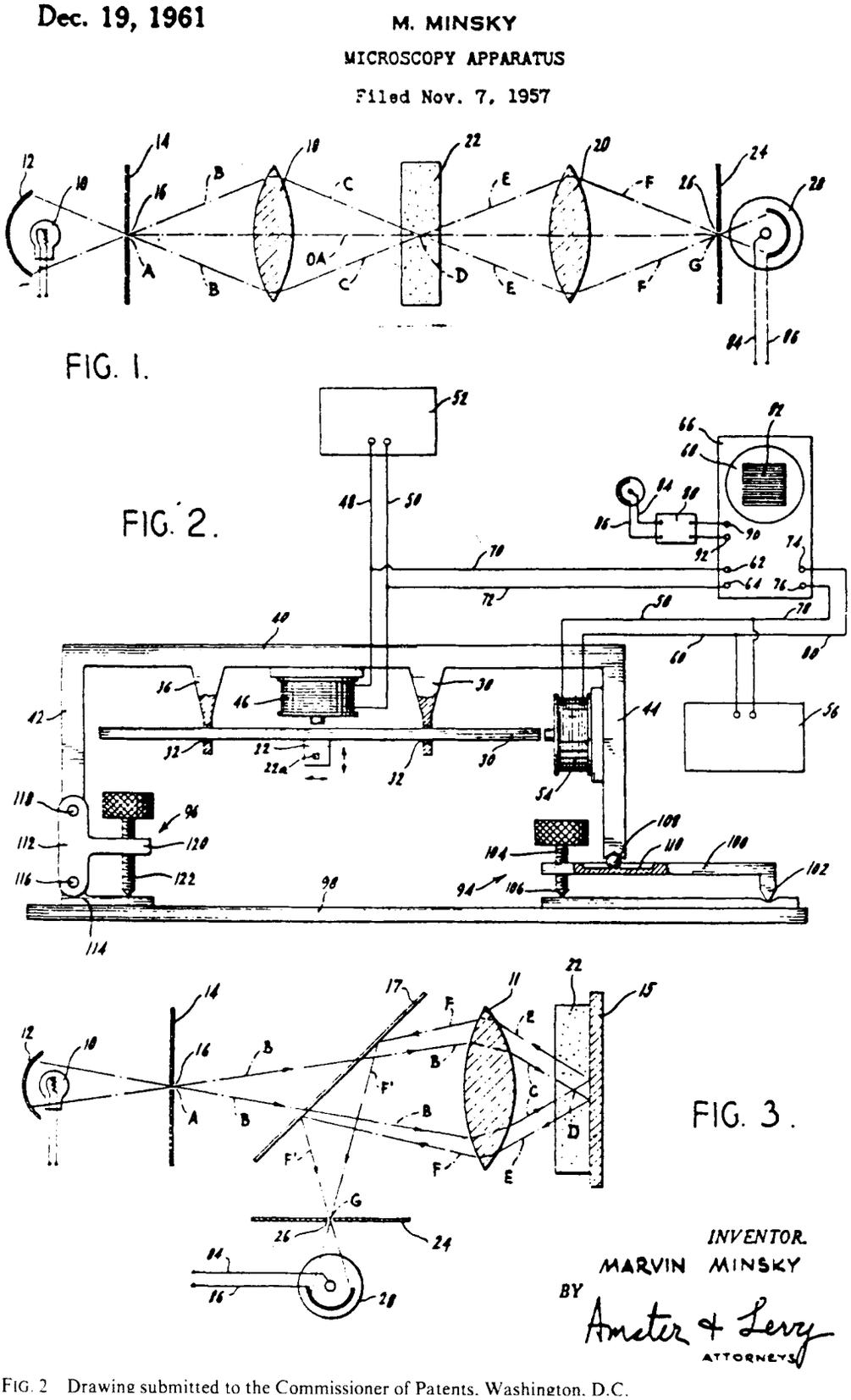


FIG. 2 Drawing submitted to the Commissioner of Patents, Washington, D.C.