unchanged. Since the volume of the fiber can be assumed to remain more or less constant, these observations show that the coefficient of birefringence of the fiber must remain constant and be independent of fiber length.

Furthermore, Fig. 2 shows that the area of the "ellipse" of the stimulated and contracted fiber is essentially no different from (or only very slightly less than) the area of the unstimulated fiber at the same length. Interestingly, my conclusion that the birefringence of muscle under isometric contraction is unchanged for that muscle at the same length at rest agrees with what Bozler and Cottrell found with snail smooth muscle, although differing from theirs and others' earlier findings on striated muscle.

From these observations, I postulated that the coefficient of birefringence (i.e., retardation per unit thickness, or the state of folding of its polypeptide chain in a cross-striated muscle fiber) was independent of fiber length, and also whether it was isometrically contracted or not. In other words, contraction and length change of muscle could not be explained by changes in folding of its polypeptide chains.

That was a few years before A.F. Huxley and R. Niedergerke and Hugh Huxley and Jean Hanson, through observations of the A- and I-band lengths in contracting striated muscle and myofibrils, proposed the mechanism of muscle contraction by the mutual sliding of actin and myosin filaments. Prior to, and even for a few years after, their work, muscle was believed by many investigators to contract, not by sliding filaments,[1] but by folding of its polypeptide chains, much the same way that an extended rubber band shortened by folding of its isoprene chains. Associated with folding of polymer chains, one expected a substantial loss of birefringence as summarized extensively for muscle fibers by W.J. Schmidt (1937). Nevertheless, by the early 1940s, there appeared several conflicting publications regarding whether the birefringence, in fact, did or did not change during contraction of muscle.

Sadly, I never did publish my findings recorded here, since Dr. Kamada, my supervisor for the project, argued that "One cannot learn about the contraction mechanism of muscle by studying cross-striated muscle since they have such a complicated, striated structure. Instead, you must learn to isolate single smooth muscle cells and work with them!" In retrospect, it was an ironic statement indeed, in view of what the striations in skeletal muscle were to reveal in 1954. However, Dr. Kamada himself never learned of those revelations since he passed away in 1948 from terminal cancer.

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[1] See Stephens (1965) for experiments using UV microbeam disruption of sarcomere regions in glycerinated myofibrils. Through these tests, he was able to convince the skeptics and rule out all but the sliding filament theory.

COVER-SLIP THICKNESS GAUGE

Shinya Inoue*

(Article prepared by author in August 2006, based on the actual gauge)

While acting as a teaching assistant in Kenneth Cooper's Cell Biology course at Princeton University in 1950, I learned that to use a microscope with high NA (numerical aperture) objective lenses, one needs to select cover slips of the proper thickness in order to get the best image. The reason was that high NA microscope objective lenses are designed to provide an image with minimum aberration only when used with the designated cover slip. In most cases, the designated thickness is 0.17 mm, coupled with the proper immersion medium and correct optical tube length, assuming the specimen is sitting close to the cover slip (see e.g., Shillaber, 1944; Inoue and Spring, 1997).

0.17-mm-thick cover slips are available commercially in boxes designated as #1.5, but those boxes usually contain cover slips whose thicknesses range from approximately 0.15 mm to 0.18 mm. In order to examine the specimen critically at high NA, one then needs to select those that do not deviate from 0.17 mm by more than +/–0.005 mm, or just a few micrometers.

To determine such thicknesses, one can use a machinist's precision caliper micrometer. However, using a caliper micrometer is somewhat cumbersome, and also one needs to scrupulously clean the contact faces of the micrometer. In order to avoid using a precision caliper micrometer altogether and to simplify the process, I designed the following cover-slip gauge that is easy to use and involves no moving parts except for the cover slip itself, which acts as the pointer for the thickness scale.

The gauge (Figs. 1 and 2) contains a narrow horizontal slit (or "gap") between two horizontally oriented edges of stainless steel razor blades. The upper blade is mounted directly onto an upright base plate, while the lower blade is mounted slightly further out by the presence of an underlying shim (Fig. 2; in the photograph, the two half-length stainless steel safety razor blades are hidden behind the brass plates which secure them in place against the thick base plate to the far left). The thick base plate is recessed behind the gap between the two blades (to the left in the figures), so that part of a cover slip which is dropped into the gap between the two blades protrudes into this narrow recess. Since the main bulk of the cover slip remains to the right of the blades, that side of the cover slip is heavier and tilts down until its tilt is constrained by the two knife edges.

The degree of tilt of the cover slip is determined by the vertical distance between the two blades and their horizontal offset (the thickness of the shim), with the lower knife edge

*Unpublished studies (1951).

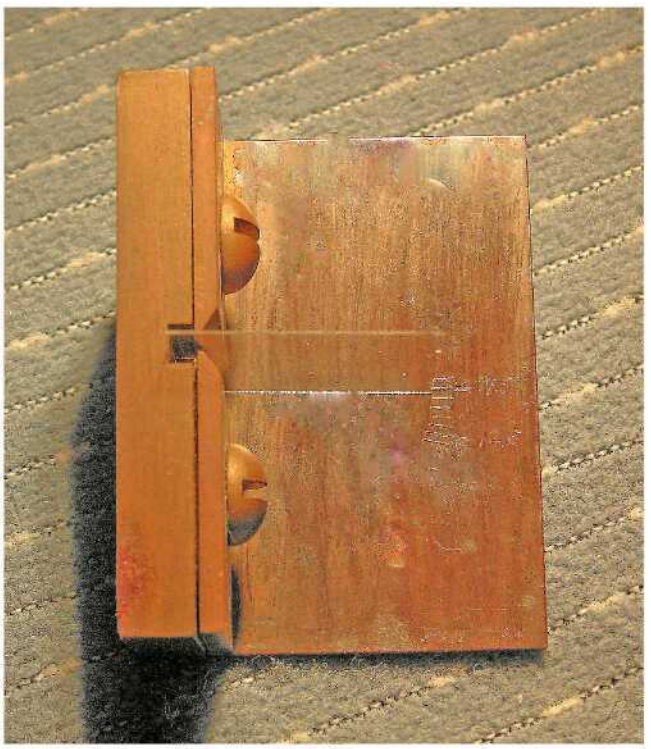

Fig. 1. Photograph of cover-slip gauge.

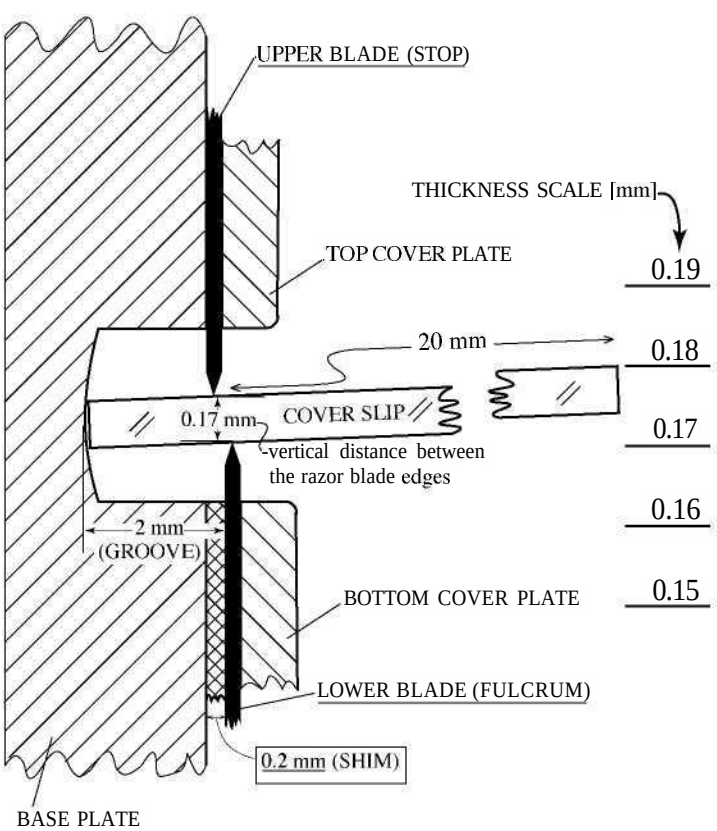

Fig. 2. Schematic of the lever system.

acting as the fulcrum, while the upper one acts as the stop that limits the tilt of the cover slip. Thus, in the gauge, the cover slips tilt differently depending on their thickness.

As shown in the figures, the actual thickness of a particular cover slip is read off from the scale inscribed on a second plate oriented at 90° to both the base plate and the slit between the razor blades.

In detail, the vertical distance between the two edges of the blades is set to 0.17 mm so that a cover slip of that thickness settles horizontally. Cover slips, whose thicknesses deviate from 0.17 mm, tilt away from the horizontal by amounts determined by their thickness and by the offset distance between the two knife edges. The shim behind the lower blade is 0.2 mm thick and defines the horizontal offset between the two blades. Since the horizontal offset between the two knife edges is 0.2 mm and the left edge of the cover slip drops into the 2 mm recess in the base plate, the right edge of a 22-mm square cover slip tips by $(22 - 2)/0.2$, or 1 mm for every 0.01 mm deviation in thickness (Fig. 2). Thus, the thickness of the cover slip, read off from the scale at the distal tip of the cover slip, is magnified 100 times.

This gauge, made in March 1951, is still used in our laboratory today after half a century. Without the fear of contaminating the surface of a carefully cleaned cover slip, its thickness is determined simply by dropping an edge of the cover slip into the gap while holding the gauge tipped counterclockwise. With the gauge brought back upright (as seen in Fig. 1), the cover slip comes to rest on the fulcrum- and stop-blades so that the scale at the distal edge of the cover slip directly indicates its thickness. This gauge is easy to use, works quickly, and with surprising accuracy.

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