For the purposes of our study it is essential to measure the thickness of the water layer sandwiched between the coverslips within which the microtubules are produced. The experimental setup is shown in Figure. 1. The indices of refraction of the glass coverslips and water are n_1 and n_2, respectively. The measurement is accomplished by focusing first on the bottom of the bottom coverslip to establish an origin (Figure 1a), obtained by reading the position of objective from the confocor 2 software. Then we focus on the top of the bottom coverslip and the bottom of the top coverslip (Figure 1b), noting the readings for each. Because we are using a water-immersion objective the difference between the latter two measurements directly defines the thickness of the water layer. To demonstrate this we begin by describing a measurement of the thickness of a glass coverslip. The difference between the indices of refraction of the glass and water causes the distance readout from the software to differ from the physical distance measured through the glass. Figure 2a illustrates the geometrical configuration for a cover slip of thickness *d*. We focus the laser until we see the first reflective spot from the bottom of the cover slip. We then focus the laser to find the second spot from the top of the cover slip. The distance of these two laser spots is the image thickness of the cover slip. The image distance *D* in Figure. 2a is given by

$$D = y \cot\theta_1 \tag{1}$$

Where

$$y = d \tan\theta_2 \tag{2}$$

Inserting (2) into (1), we obtain

$$D = d \tan\theta_2 \cot\theta_1 = d \frac{\sin\theta_2}{\cos\theta_2} \frac{\cos\theta_1}{\sin\theta_1} \tag{3}$$

From Snell's law

$$n_1 \sin\theta_1 = n_2 \sin\theta_2 \tag{4}$$

By substituting $\sin\theta_2$ with $n_1 \sin\theta_1 / n_2$

$$D = d \frac{n_1}{n_2} \frac{\sin\theta_1}{\cos\theta_2} \frac{\cos\theta_1}{\sin\theta_1} = d \frac{n_1 \cos\theta_1}{n_2 \cos\theta_2} = d \frac{n_1 \cos\theta_1}{n_2 \sqrt{1 - \sin^2\theta_2}}$$

$$= d\frac{n_1 \cos\theta_1}{n_2\sqrt{1-\left(\frac{n_1 \sin\theta_1}{n_2}\right)^2}} \qquad (5)$$

In our actual experiments the incident laser is perpendicular to the cover slip, which means $\theta_1 = 0$ we have $\sin\theta_1 = 0, \cos\theta_1 = 1$. So we obtain $D = dn_1/n_2$, which means $d = Dn_2/n_1$. Hence, measurement of the thickness of the glass coverslip using a water immersion objective requires a correction for the difference between the indices of refraction of water and glass.

In our study, however, we measure the thickness of a water layer sandwiched between two coverslips as illustrated in Figure 2b. The image distance of the laser spot at the bottom of the top cover slip is D_1, while the image distance of the laser spot at the top of the bottom cover slip is D_2. D_1 is given by

$$D_1 = y_3 \cot\theta_1 \qquad (6)$$

Here we have

$$y_3 = y_1 + y_2 = d_1 \tan\theta_1 + d_2 \tan\theta_2 \qquad (7)$$

Then,

$$D_1 = (d_1 \tan\theta_1 + d_2 \tan\theta_2) \cdot \cot\theta_1 = d_1 + d_2 \tan\theta_2 \cdot \cot\theta_1 \qquad (8)$$

From previous results, when $\theta_1 = 0$ we have

$$d_2 \tan\theta_2 \bullet \cot\theta_1 = d_2 n_1 / n_2$$

So finally $D_1 = d_1 + d_2 n_1 / n_2$, this gives us $d_1 = D_1 - d_2 n_1 / n_2$. Define $D_2 = d_2 n_1 / n_2$ so that $d_1 = D_1 - D_2$. As seen in Figure 2b D_2 is just the apparent image distance when the laser is focused at the top of the bottom cover slip, which can be directly read during the experiment. Thus the distance measured directly from the fine focus knob of the microscope yields the thickness of the water layer without a correction for index of refraction.

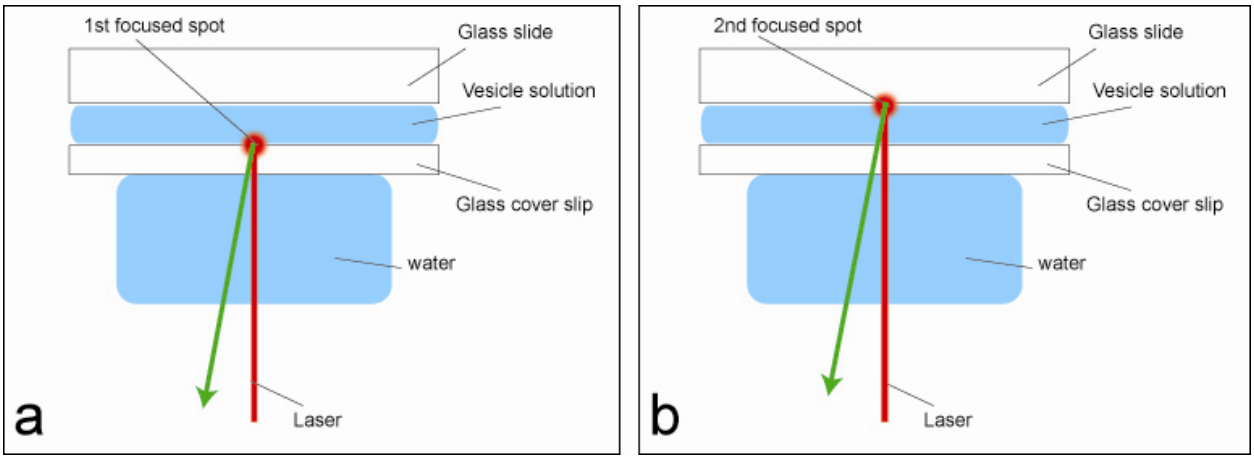

Figure 1 Setup for measuring the gap distance between cover slip and the glass slide. (a) Measuring the distance of the first focused laser spot. (b) Measuring the distance of the second focused laser spot.

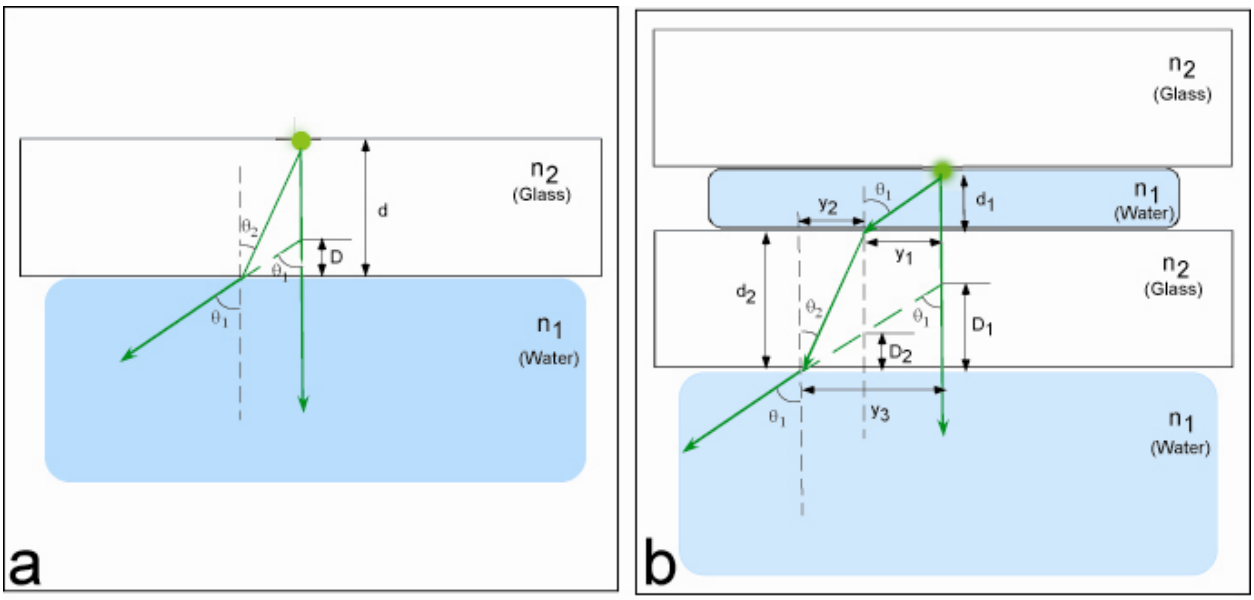

Figure 2 (a) Refraction of rays through a cover slip. The location of the virtual image of the laser reflective spot at the top surface of the cover slip is determined by the intersection of ray R_0 and the backward extension of ray R_1. **(b)** Refraction of rays through a cover slip and a layer of water sandwiched between 2 cover slips. The location of the virtual image of the laser reflective spot at the bottom surface of the second cover slip is determined by the intersection of ray R_0 and the backward extension of ray R_1

Reference: M. P. Silverman Eur J Phys 11(1990) 366-371