Simultaneous measurement of water flow velocity with fluorescent and speckle imaging technique

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ABSTRACT- The average velocity of water flow has been simultaneously measured with fluorescent and speckle imaging methods. The measured velocities with two methods are in good agreement with each other and it confirms that the speckle imaging method can be used as a confident method to measure the velocity of water flow in a dry leaf. Also the velocity of water flow through thick and thin xylems of a leaf can be measured with speckle imaging method and the ratio of thick xylem's radius to thin xylem's radius can be estimated with this method. Also the ratio was measured by monitoring the cross section of the leaf. The two measurements were in agreement.

KEYWORDS: laser, speckle, fluorescent, velocity, plant, imaging.

I. INTRODUCTION

Water flow within plants plays a crucial role in the exchange of nutrients and signal messengers between the different plant organs. Up to now, several methods have been employed in order to measure the velocity of water flow in plants. The heat pulse velocity technique uses heat as a tracer [1]. In this method heater probes and temperature sensors have to be inserted into a stem. By analysis of received information from heater probes and temperature sensors, the velocity of water flow in the stem is measured. Other technique to assess water velocity in stem is the injection of radioactive tracers into the xylem and the use of promoters to measure the velocity of water flow in xylems of a stems [2]. Nuclear magnetic resonance (NMR) imaging is also used to measure water velocity in plants. In this method, spatial information from NMR signals can be extracted using NMR imaging. The strongest signal that can be detected id the 3H NMR water signal, allowing visualization of water movement in plants [3-5]. X-ray micro-imaging technique to measure water velocity, employs phase contrast imaging of X-ray beams, which are transformed into visible light and are photographed by a charge coupled device camera [6,7]. Fluorescent imaging technique measures the velocity of water flow by following dye molecules as a tracer [8-11]. The last technique which has been recently proposed is speckle imaging technique [12]. In this method, by analysis of speckle images of a plant, the velocity of water flow is measured. Speckle technique is a nondestructive method to measure the velocity of water flow in plants because no extra material or device is inserted into the plant. The last method seems to be a proper method to measure the velocity of sap flow in plants however the measured velocity with this method hasn't been tested with another standard method.

In this paper, we will compare velocity of water flow which is measured by the speckle imaging method with velocity of water flow which is measured by the fluorescent imaging method. So we are going to simultaneously measure the velocity of sap (water) flow with the speckle imaging method and fluorescent imaging method.

In fluorescent imaging method a plant is irrigated with a solution of water and dye molecules. As the solution rises up through the xylems of the plant, the dye molecules can be followed as a tracer: when a laser beam is irradiated on the surface of the plant the dye molecules, which is located in the xylem, emit fluorescent light. By detection of fluorescent

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light, the position of dye molecules can be monitored and water flow in xylems can be traced. So the velocity of water flow can be measured.

In speckle imaging method a laser beam is irradiated onto the leaf of a plant. The light is partially reflected from the plant surface and the rest penetrates into the leaf. Micro particle structures in the leaf backscatter the laser light and the scattered light passes through the water that is in the tissue of a leaf and forms a speckle pattern on a CCD camera. Because of random movements of suspended particles in the water, speckle pattern is changed continuously over time. Faster changes in speckle pattern are obtained by high amount of water content. When the leaf losses its water and the membrane of plant cells shrinks, changes in speckle pattern are decreased. If we irrigate the dry leaf, water rises up through xylems of the leaf and makes the faster changes in speckle pattern. So the speckle pattern is changed due to water absorption. By analysis of speckle pattern we can trace the water flow in the leaf and measure the velocity of water flow.

In section 2 principle of fluorescent and speckle imaging are discussed. In section 3 the experimental setup and procedure is described. Data analysis is presented in section 4 and finally the results are compared in section 5.

II. PRINCIPLE OF THE METHOD

A. *Fluorescent imaging technique*

Water rises up through xylems of a plant. If we irrigate a plant with a solute of water and dye, we can monitor water flow by tracing dye molecules (Fig. 1). To trace dye molecules, a laser beam is irradiated on a leaf of a plant. The light penetrates into the leaf and is scattered from dye molecules. The dye molecules scatter fluorescent light. The scattered fluorescent light passes through a filter to record a fluorescent image of a leaf on a CCD camera. The CCD camera shows the fluorescent image of a leaf. As dye molecules rise up through the xylems of the leaf, several fluorescent images of the leaf is captured.

By analysis of fluorescent images, the position of dye molecules can be visualized. If we focus on a point of the leaf and plot the diagram of fluorescent intensity of the point versus time, we can obtain the time of reaching the dye molecules (among water) to that point. For points A, B …, shown in Fig. 1, we plot the diagram of fluorescent intensity versus time and obtain the time of reaching water to the points which are named t_{fA}, t_{fB} … For two separated points like A and D on the leaf, we can obtain t_{fA} and t_{fD} and the distance between these two points (d) so the velocity of water flow between points A and D can be estimated as follows:

$$V_{fluo} = \frac{d}{t_{fD} - t_{fA}} \quad (1)$$

where V_{fluo} is the velocity of water flow which is measured with fluorescent imaging method.

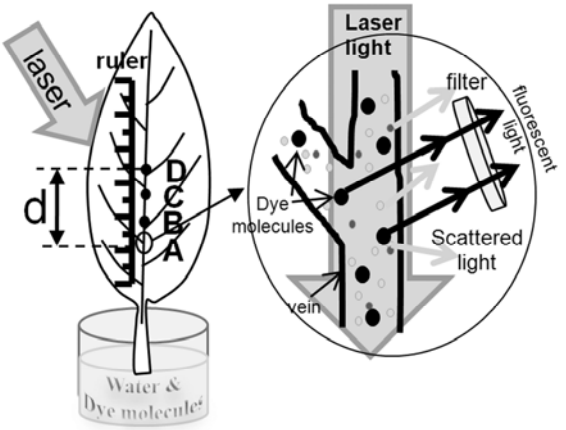

Fig. 1. In fluorescent imaging technique, a leaf is irrigated with the solution of water and dye molecules and a laser light is irradiated onto the leaf. By tracing dye molecules, water flow in the leaf can be monitored.

B. *Speckle imaging method*

As shown in Fig. 2, when a laser beam is irradiated on a surface of a leaf, the light penetrates into the leaf and is scattered from surface and inner structures of the leaf. The scattered light forms a speckle pattern on a CCD camera. The speckle pattern is due to two parts of the leaf: solid parts (like membrane of xylem or phloem) and liquid parts (like water and sap flow in xylem and phloem). The speckle pattern of solid parts of the leaf doesn't change over the time because

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the solid parts of the leaf are fixed during the experiment, But the speckle pattern of liquid parts changes over the time because the liquid moves over with the time, So the changes in speckle pattern of a leaf are due to liquid (water) parts of the leaf. Faster changes in speckle pattern are obtained by high amount of water content in the leaf.

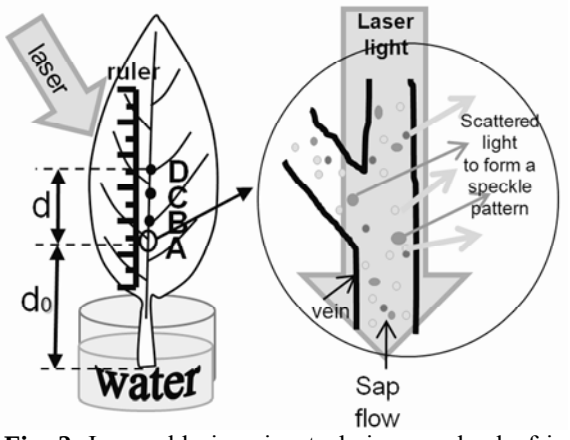

Fig. 2. In speckle imaging technique, a dry leaf is irrigated with water and a laser light is irradiated onto the leaf and the scattered light forms a speckle image of the leaf. By analysis of the speckle image, water flow in the leaf can be monitored.

Changes in speckle pattern can be estimated by a parameter called μ [12]. μ is defined as follow:

$$\mu = \frac{\langle [I_{ref}(x,y) - I_{def}(x,y)]^2 \rangle}{\langle I_{ref}^2(x,y) \rangle - \langle I_{def}^2(x,y) \rangle} \qquad (2)$$

where $I_{ref}(x,y)$ is the speckle intensity value of a point (x,y) at time t=0s. $I_{def}(x,y)$ is the speckle intensity value of point (x,y) in another time (t≠0s) and <…> denotes the ensemble average. This parameter is directly related to the difference between speckle patterns. The high μ shows the high difference between speckle patterns and the low μ shows the low difference between speckle patterns, so high μ shows the high amount of water content in the leaf and vice versa. A dry leaf which losses its water has a less μ than a fresh leaf [12]. If we irrigate a dry leaf, the water content of the leaf is increased. Before irrigation its μ parameter would be low and after irrigation its μ parameter is increased.

By irrigating a dry leaf, water rises up through xylems of the leaf. At first the μ parameter of lower part of the leaf is increased (because of existence of water in this part). Then the μ parameter of upper part of the leaf is increased (because water reaches the upper part of a leaf). As seen in Fig. 2, the lower part of the leaf is shown by point A and the upper part by point D. By analysis of diagram μ versus time, we can estimate the time of reaching water to these points. We name the time of reaching water to the point A equal to t_{sA} and the time of reaching water to the point D equal to t_{sD}. t_{sA} and t_{sD} are the time of reaching water to the points A and D measured by speckle imaging method.

The velocity of water flow between points A and D can be calculated as follows:

$$V_{spe} = \frac{d}{t_{sD} - t_{sA}} \qquad (3)$$

where d is the distance between points A and D. V_{spe} is the velocity of water flow between points A and D which is measured with speckle imaging method.

C. *Simultaneous method of fluorescent and speckle imaging*

A dry leaf is employed to simultaneously measure the velocity of water flow with fluorescent imaging method and speckle imaging method. R590 (479 g/mol) was used as a dye tracer. It is dissolved in ethanol (4% of R590 and 96% of ethanol). A solution of R590 in distilled water is employed to irrigate a dry leaf of *Eriobotrya japonica* plant. A green laser is irradiated on the leaf and the scattered light from the leaf is recorded. One section of scattered light passes through a filter to form a fluorescent image on a CCD. Another section of scattered light forms a speckle pattern on another CCD. Thus two simultaneous fluorescent and speckle images of the leaf are recorded. By analysis of these images the velocity of water flow with two simultaneous methods of fluorescent and speckle imaging is measured.

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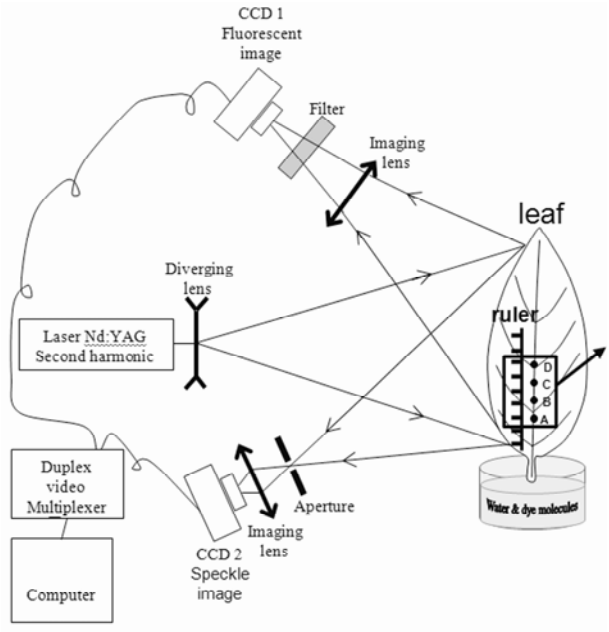
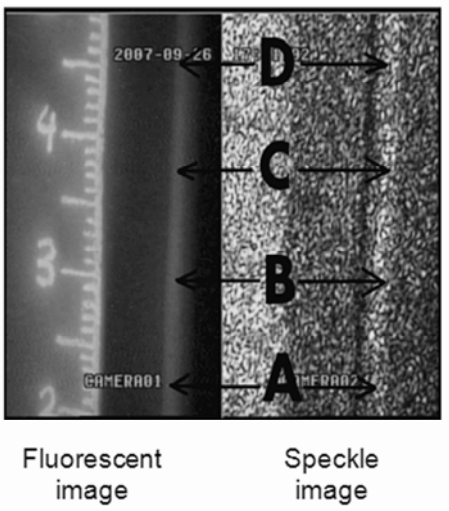
III. EXPERIMENTAL SETUP

Experimental setup is shown in Fig. 3. The second harmonic of Nd:YAG laser is employed to produce a green CW laser beam with λ=532nm.



(a)



(b)

Fig. 3. (a) Experimental setup and (b) a simultaneous fluorescent and speckle image of a leaf which is captured by two CCDs.

The laser beam is irradiated on a leaf of *Eriobotrya japonica* plant which had been cut 3 hours before the beginning of the experiment. The laser beam is incident on the leaf and is reflected and scattered from it. One section of scattered light passes through an imaging lens and a filter to form a fluorescent image of the leaf on CCD1 camera. Another section of scattered light passes through a pine hole and an imaging lens to form a speckle image of the leaf on CCD2 camera.

Our CCD cameras operate at 25 frames per second (fps) and 768×576 pixel. During our experiment, the rate of frame grabbling could be changed according to our requirements. Duplex Video Multiplexer simultaneously records images from CCD1 and CCD2 and transforms them to a computer via a NIPCI 1411 image acquisition card. Image processing is done by LabWiew which is powered by its image processing module, NI VISION DEVELOPMENT.

A typical image which was captured from two CCDs is shown in Fig. 3. The left part of the image is the fluorescent image of the leaf captured by CCD1 and the right part of the image is the speckle image of the same leaf captured by CCD2.

IV. 4. DATA ANALYSIS

A. *Recording simultaneous fluorescent and speckle images*

A leaf of *Eriobotrya japonica* plant which had been cut 3 hours before the beginning of the experiment was put as a sample in experimental setup at Fig. 3. The stem of the leaf was put in an empty pot. A ruler which has been marked by dye material was stuck on the leaf as shown in Fig. 3. The solution of R590 and distilled water was poured in the pot. Simultaneous fluorescent and speckle images of the leaf were captured over the time by two CCDs. CCD1 captures the fluorescent images of the leaf by the rate of 10 fps and CCD2 simultaneously captures speckle images of the leaf by the same rate.

Figure 4 shows four simultaneous fluorescent and speckle images of the leaf which have been recorded in different times. Fig. 4a has been recorded before the irrigation, in the beginning of the experiment (at t=0s). The leaf was irrigated with the solution of R590 and distilled water at t=18s. Then the images of 4b, 4c and 4d were taken at times 31s, 37s and

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43s. Rising up of the liquid through the vein of the leaf is illustrated in the fluorescent images of Fig. 4.

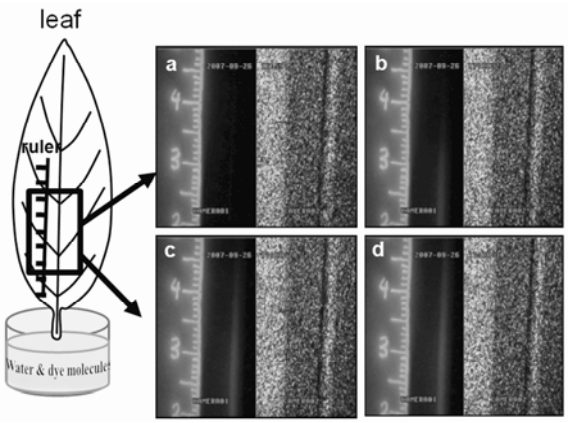

Fig. 4. Captured images from two CCDs in setup at times (a) 0s, (b) 31s, (c) 37s and (d) 43s.

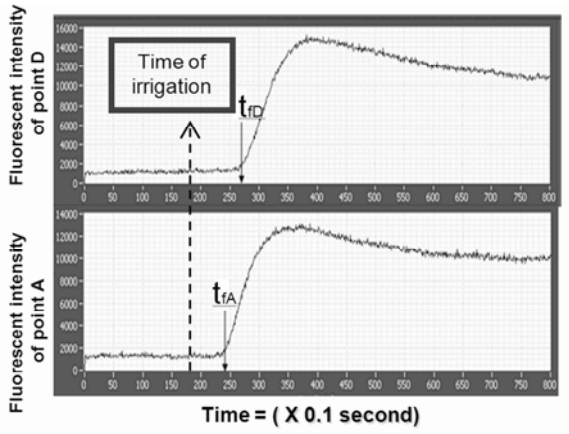

Fig. 5. Diagrams of fluorescent intensity versus time for points A (lower area) and D (upper area) of the leaf. The leaf was irrigated at t=18s and t_{fA} (t_{fD}) is the fluorescent start time for poit A (D).

B. *Velocity measurement with fluorescent imaging method*

In order to measure the velocity of water flow with fluorescent imaging method, the time of water reaching (among dye molecules) to the points A, B… should be obtained (Fig. 1). For this purpose, by analysis of captured images from CCD1, the diagram of fluorescent intensity versus time for points A, B… has been plotted as shown in Fig. 5. In these diagrams at the beginning of the experiment (t=0s) the fluorescent intensity is zero. At time t=18s the leaf is irrigated with the solution of water and dye. Then at time t_{fA} the fluorescent intensity is increased at lower diagram and at time t_{fD} the fluorescent intensity is increased at upper diagram. t_{fA} is the time in which the dye molecules reach point A and t_{fD} is the time in which the dye molecules reach point D. At last, the fluorescent intensity reaches its maximum value at time t>40s. Therefore t_{fA}, t_{fB} … can be obtained from the diagrams of fluorescent intensity for points A, B…. The results are shown in table 1. As expected, table 1 shows that $t_{fD} > t_{fC} >$ … The distance between points A and D is measured by a ruler which is shown in Figs. 3 and 4. Thus from equation 1 the velocity of water flow with fluorescent imaging method is obtained as follow:

$$V_{fluo} = \frac{d}{t_{fD} - t_{fA}} = \frac{2.4\ cm}{3\ s} = 29 \pm 2\ m/h \quad (4)$$

Table. 1. The time of reaching water to different points of a leaf which are measured by fluorescent imaging method (t_f) and speckle imaging method (t_s).

	Point A	Point B	Point C	Point D
t_f (Second)	24.0 ± 0.2	25.0 ± 0.2	26.0 ± 0.2	27.0 ± 0.2
t_s (Second)	26.0 ± 0.2	27.0 ± 0.2	27.5 ± 0.2	29.0 ± 0.2

C. *Velocity measurement with speckle imaging method*

In order to measure the velocity of water flow in xylems of a leaf, the time of reaching water to points A, B … should be measured with speckle imaging method. To measure the time of reaching water to each point, the diagram of μ parameter versus time should be plotted. By analysis of the diagrams, the time can be measured. Using speckle images of the leaf, the diagram of μ parameter versus time, recorded by CCD2, is plotted for each point. The points are actually small sections of an image so that μ parameter for these sections is calculated. To calculate μ parameter, the speckle pattern of each point of the leaf, which is recorded at time "t", was compared with the speckle pattern recorded at time "t+0.25s". From Eq. 2, μ parameter of each point at time "t" is obtained. Thus the diagram of μ parameter versus time for each point can be plotted. Fig. 6 shows the diagram of μ parameter versus time for point A and D. Fig. 6a is the diagram of μ parameter for point D

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(upper area of the leaf) and Fig. 6b is the diagram for point A (lower area of the leaf). Fig. 6c shows two diagrams of points A and D in one diagram. Fig. 6c illustrates that two curves are shifted in respect to each other.

1) Analysis of the diagram of μ parameter versus time

Figure 6 shows that at the beginning of the experiment (t=0s), μ parameter is in its minimum value because the leaf, which has been cut 3 hours before the experiment, losses water, so the changes in speckle pattern of the leaf is low. At time t=18s the leaf is irrigated. Irrigation makes the leaf to absorb water. Diagrams of fluorescent intensity in Fig. 5 show that points A and D absorb water a little later than the time of irrigation. The irrigation is done at time t=18s while the absorption for point A (D) starts at time t=24s (t=27s) and ends around t=40s. Thus these fluorescent diagrams illustrate that during the time 24s<t<40s the section of the leaf located between points A and D, absorbs water and it is saturated for the time t>40s.

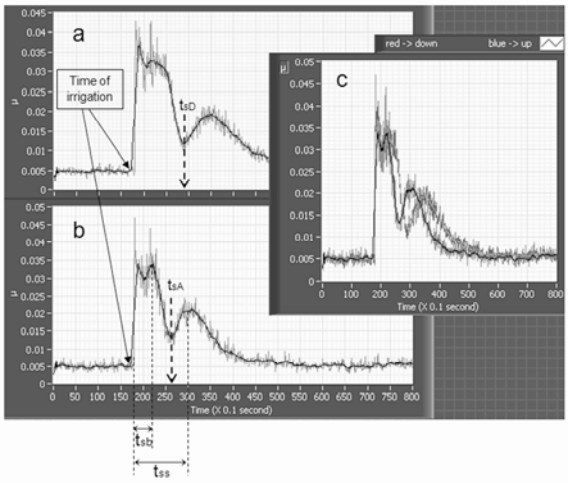

Fig. 6. Diagram of parameter μ versus time for (a) upper area (b) lower area (c) upper and lower area of a leaf with 24 mm separation. The leaf was irrigated at t=18s.

Speckle diagrams in Fig. 6 show that μ parameter is increased from the time of irrigation (t=18s) till t=40s. The μ parameter is decreased at t>40s because the leaf is saturated and no changes in water content are made.

There are three separated peaks in diagrams of Fig. 6. First peak (the first peak from the left) is created at the time of irrigation (t=18s). The first peak in Fig. 6a is overlapped with the first peak in Fig. 6b while the second and third peaks are shifted (as seen in Fig. 6c). The second and third peaks are formed at time 24s<t<40s. As it was shown from fluorescent diagrams of Fig. 5, points A and D of the leaf absorb water between times 24 to 40 seconds after irrigation. Therefore, the second and third peaks in Fig. 6 are created due to water absorption.

The first peak in Fig. 6 is formed at time t=18s. From fluorescent diagram of Fig. 5 at this time points A and D have not yet absorbed water yet, so the first peak in Fig. 6 is not created due to water absorption. It seems that the first peak is formed due to microstructure's movement at the leaf which is caused by irrigation. To prove this idea, we have done another experiment as follows:

A leaf of *Eriobotrya japonica* plant, which had been cut 3 hours before the experiment, was placed in the setup of Fig. 3. Simultaneous fluorescent and speckle images of the leaf were captured over time by CCDs. Image recording started at time t=0s. Similar to previous experiment the leaf was irrigated with the solution of water and R6G (as a dye tracer) at t=18s. Fig. 7 shows four captured images at various times. The image of Fig. 7a was recorded at time t=0s and images of Figs. 7b, 7c and 7d were captured at t=31s, 37s and 43s. From fluorescent images of Fig. 7, it is illustrated that water hasn't arrived to the considered section and the xylems of this section haven't absorb water yet. It could be because of slow movement of water through xylems of the leaf. Thus it is expected that in diagrams of μ parameter versus time, the second and third peaks, which are caused by water absorption, do not appear.

Figure 8 shows the diagram of μ parameter versus time for the upper area (Fig. 8a) and the lower area (Fig. 8b) of the leaf. The first peak is just appeared in diagrams of Fig. 8. As expected, the second and third peaks, which are caused by water absorption, do not appear

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here. Thus it shows that the second and third peaks in diagram of μ parameter versus time are caused by water absorption.

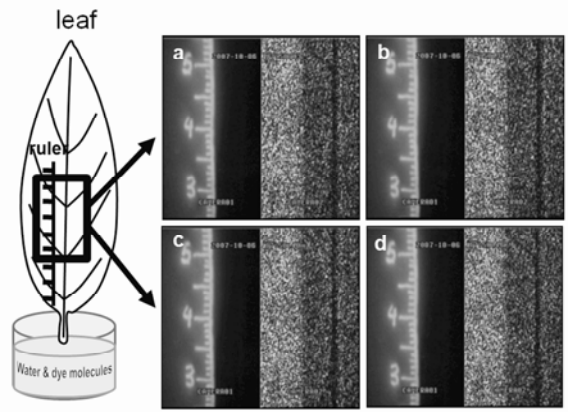

Fig. 7. Captured images from two CCDs in setup at times (a) 0s, (b) 31s, (c) 37s and (d) 43s. Fluorescent images in left hand side, illustrate that water has not arrived to the considered section

On the other hand, the first peak, which could be caused by microstructure's movement of a leaf due to irrigation, still exists. Similar to Fig. 6, the first peak of diagram 8a is approximately overlapped with the first peak of diagram 8b. This is shown in Fig. 8c. Therefore the first peak of Fig. 6 seems to be due to leaf's movement during irrigation and the second and third peaks due to water movements through xylems of the leaf. The difference between second and third peaks is tried to be interpreted in the following subsection.

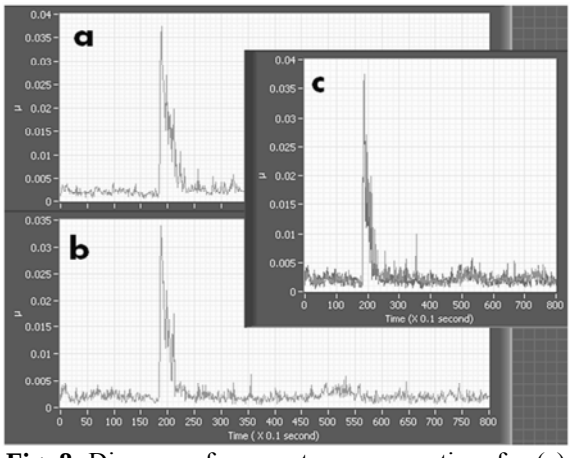

Fig. 8. Diagram of parameter μ versus time for (a) upper area (b) lower area (c) upper and lower area of a leaf with 24 mm separation. Although the leaf was irrigated at t=18s, Water has not arrived to the considered section on the leaf of Fig. 7.

2) Water velocity in thick and thin xylems of a leaf with speckle imaging method

A stem or a vein of a leaf contains a number of thick and thin xylems (mostly 2-100 μm in diameter) [13]. The velocity of water flow through these xylems is related to their diameters [14] and obeys the following equation:

$$V = \frac{R^2 \Delta p}{8 \mu_1 L} \qquad (5)$$

where V is the longitudinal velocity of water flow through a xylem, R is radius of the xylem, Δp is difference of pressure (tension) from beginning to end of a xylem, μ_1 is dynamic viscosity of sap (water) flow through the xylem and L is length of xylem. Eq. 5 expresses that the velocity of water flow in xylem with bigger radius is more than the velocity of water flow in xylem with smaller radius.

The second peak in diagram of μ parameter seems to be due to water flow in xylems with big radius and the third peak is due to water flow in xylems with small radius. Fig. 6c shows the shift of the second peak as $\Delta t_2 = 2 \pm 0.1$s and the shift of the third peak as $\Delta t_3 = 5 \pm 0.1$s. The distance between points A and D on the leaf is d=2.4 cm, so the velocity of water flow due to the shift of the second peak (the velocity of water flow in xylems with big diameter-V_b) is:

$$V_b = \frac{d}{\Delta t_2} = 43 \text{ m/h} \qquad (6)$$

and the velocity of water flow due to the shift of the third peak (the velocity of water flow in xylems with small diameter-V_s) is:

$$V_s = \frac{d}{\Delta t_3} = 14.5 \text{ m/h} \qquad (7)$$

Thus it seems that the velocity of water flow through thick and thin xylems of a leaf can be measured with speckle imaging method.

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3) Obtaining the ratio of thick xylem's radius to thin xylem's radius with speckle imaging method

From Eq. (5) the velocity of water flow in thick xylems (V_b) is:

$$V_b = \frac{R_b^2 \Delta p}{8\mu_1 L} \quad (8)$$

where R_b is the radius of thick xylems. The velocity of water flow in thin xylems (V_s) is:

$$V_s = \frac{R_s^2 \Delta p}{8\mu_1 L} \quad (9)$$

where R_s is the radius of thin xylems. If we divide Eq. (8) by Eq. (9), we have:

$$\frac{V_b}{V_s} = \left(\frac{R_b}{R_s}\right)^2 \quad (10)$$

From Eq. (10) we define R_{bs} as the ratio of thick xylem's radius to thin xylem's radius:

$$R_{bs} = \frac{R_b}{R_s} = \sqrt{\frac{V_b}{V_s}} \quad (11)$$

R_{bs} can be estimated from Eq. (6) and (7):

$$R_{bs} = \sqrt{\frac{V_b}{V_s}} = 1.72 \pm 0.05 \quad (12)$$

R_{bs} can be estimated in another way: In Fig. 2 distance between point A and the beginning of the stem is d_0. Water rises up from the beginning of the stem to point A through thick xylems which it takes t_{bs}=4s (it is shown in Fig. 6b). If water rises up to point A through thin xylems, it takes t_{ss}=12s. From Eq. (11), t_{bs} and t_{ss} we can estimate R_{bs} as follow:

$$R_{bs} = \sqrt{\frac{V_b}{V_s}} = \sqrt{\frac{d_0/t_{sb}}{d_0/t_{ss}}} = \sqrt{\frac{t_{ss}}{t_{bs}}} = 1.73 \pm 0.05 \quad (13)$$

R_{bs}, which is obtained from Eq. (13) is agreed with Eq. (12). Also R_{bs} can be obtained by monitoring the cross section of the vein. It is done in the following subsection.

4) Obtaining the ratio of thick xylem's radius to thin xylem's radius by monitoring the cross section of the leaf

To monitor xylem's bundles in the vein of Eriobotrya japonica plant, a hand section were cut from the vein and viewed with a green induced fluorescent optics shown in Fig. 9.

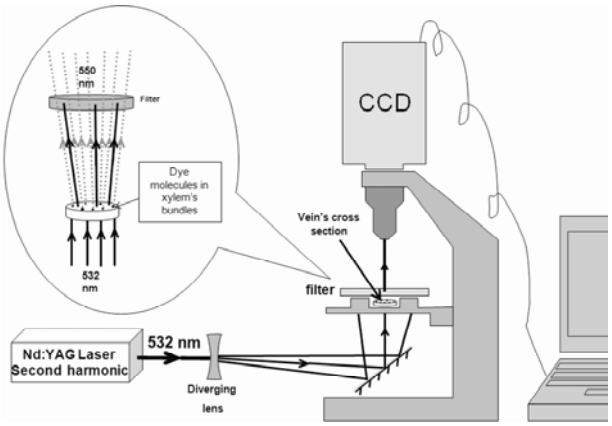

Fig. 9. Experimental setup to take a microscopical fluorescent photograph of vein's cross section

The second harmonic of Nd:YAG laser is employed to produce a green CW laser beam with λ=532nm. The laser beam is illuminated on the hand section of the vein. Dye molecules in xylem's bundles of the vein emit fluorescent light which is passed through a filter and is viewed by a biological microscope (Model: BM-22h). A microscopical fluorescent photograph of the vein's cross section is shown in Fig. 10. Dye molecules in xylem's bundles emit fluorescent light which is shown in this figure. Therefore the xylems'position in the vein of Eriobotrya *japonica* plant is monitored.

To obtain distribution of xylem's radius in the vein of our leaf, the piece was hand-sectioned with a sharp steel razor blade. Special attention was given to drawing the blade gently through the tissue to avoid crushing cells. The hand-sectioned piece was stained with blue methyl and viewed by the biological microscope. Fig. 11 shows Vein's cross section of *Eriobotrya japonica* plant stained with blue methyl. The position of xylems in Figs. 10 and 11 are the same so we can distinguish the xylem's bundles in Fig. 11 and measure the radius of each xylem. Fig. 12 shows xylem's diameter distribution in the vein of *Eriobotrya japonica*

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plant which had been placed in the setup of Fig. 3.

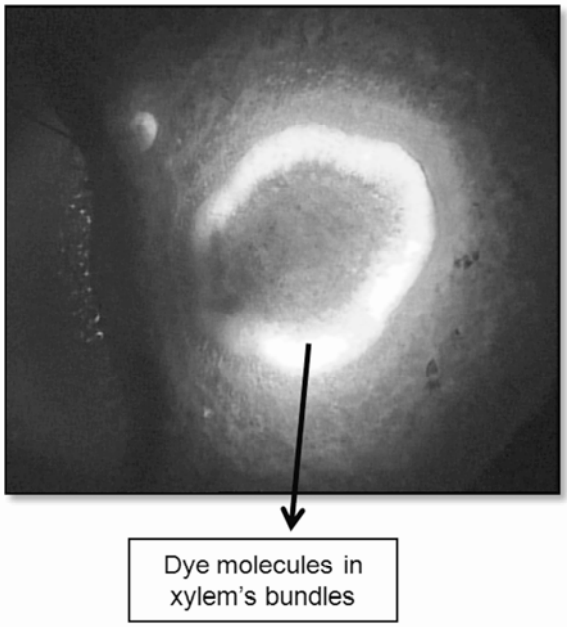

Fig. 10. A microscopical fluorescent photograph of vein's cross section of *Eriobotrya japonica plant* which is taken by setup in Fig. 10. Dye molecules are in xylem's bundles

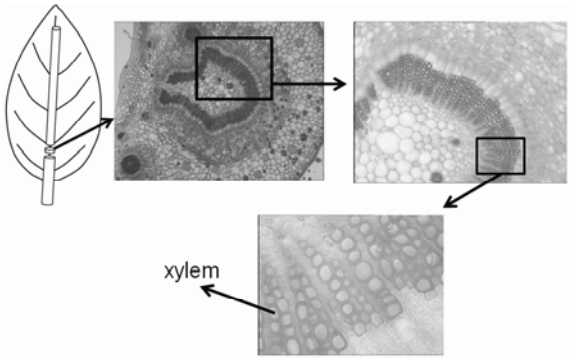

Fig. 11. Vein's cross section of *Eriobotrya japonica* plant stained with blue methyl. xylem's bundles are in blue.

Figure 11 shows two separated peaks and it means that there are mostly two xylem's diameters in the vein. The radius of thick xylems is:

$R_b = 5.6\ \mu m$

And the radius of thin xylems is:

$R_s = 3.3\ \mu m$

Thus we will have:

$$R_{bs} = \frac{R_b}{R_s} = 1.7 \pm 0.1 \qquad (14)$$

Thus the ratio of thick xylem's radius to thin xylem's radius (R_{bs}) is obtained by monitoring the cross section of the leaf and it is in good agreement with the ratio which had been obtained by speckle imaging method (Eq. (14) which is in agreement with Eq. (13) and Eq. (12)).

Therefore speckle imaging method seems to be a proper method to measure the ratio of thick xylem's radius to thin xylem's radius in a leaf, without cutting the leaf.

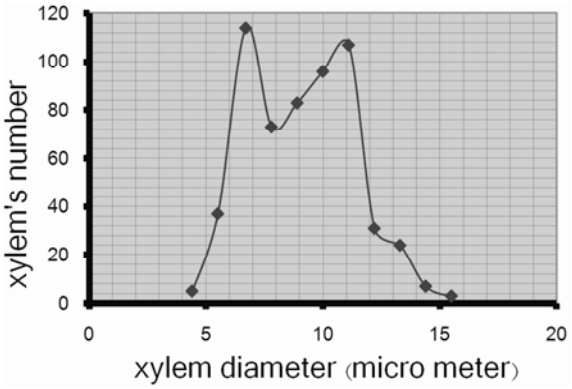

Fig. 12. Xylem's diameter distribution in the vein of *Eriobotrya japonica plant.*

5) Measuring the average velocity of water flow in xylems of a leaf with speckle imaging method and compare the result with the fluorescent imaging method

By averaging Eq. (6) and (7), the average velocity of water flow through thick and thin xylems of the leaf is obtained as follow:

$$V_{spe} = 28.8 \pm 2\ m/h \qquad (15)$$

The average velocity of water flow in xylems of a leaf can be estimated with another method of speckle imaging. In Fig. 6a (6b) the minimum point between the second peak and third peak can be considered as the average time of water reaching to point D (A) which is named t_{sD} (t_{sA}). t_{sD} is the average time of reaching water to xylems of point D which is measured by speckle imaging method. By this method, the average time of reaching water to points A, B, C and D (t_{sA}, t_{sB} …) can be measured. Table 1 shows t_{sA}, t_{sb} …. As

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expected, $t_{sD}>t_{sC}>\ldots$. Thus from Eq. (3) and table 1, the average velocity of water flow in xylems of the leaf is obtained:

$$V_{spe} = \frac{d}{t_{sD}-t_{sA}} = 29 \pm 2\ m/h \qquad (16)$$

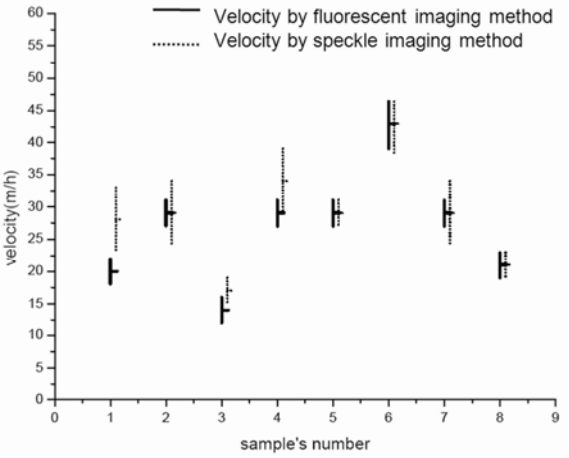

Fig. 13. The comparison of the velocity of water flow in *Eriobotrya japonica* leaf by fluorescent imaging method with the velocity by speckle imaging method for eight different samples.

Eq. (15) and (16), the average velocity of water flow with speckle imaging technique, are in good agreement with Eq. (4) which is the velocity of water flow with speckle imaging technique. The experiment has been repeated for eight different leaves of *Eriobotrya japonica* plant. The results are shown in Fig. 9. In this figure solid lines show the measured velocity with fluorescent imaging method and dashed lines show the measured velocity with speckle imaging method. In Fig. 13 the measured velocities with two methods are in good agreement with each other.

V. CONCLUSION

The average velocity of water flow has been simultaneously measured with fluorescent and speckle imaging methods. For this purpose, a dry leaf of *Eriobotrya japonica* plant was employed and irrigated by a solution of dye molecules and water. A laser beam was irradiated on the leaf and scattered from the leaf. By analysis of scattered light, the velocity of water flow in xylems of the leaf was measured with two methods. The measured velocity with speckle imaging method was equal to 28.8±2 m/h and the measured velocity with fluorescent imaging method was equal to 29±2 m/h. The experiment has been repeated for eight different leaves of *Eriobotrya japonica* plant. The measured velocities with two methods were in good agreement with each other and it confirms that the speckle imaging method can be used as a confident method to measure the velocity of water flow in a dry leaf. Also the velocity of water flow through thick and thin xylems of a leaf was measured with speckle imaging method and the ratio of thick xylem's radius to thin xylem's radius was estimated with this method. Also the ratio was measured by monitoring the cross section of the leaf. The two measurements were in a good agreement with each other and it confirms that the speckle imaging method can be used as a proper method to measure the ratio of thick xylem's radius to thin xylem's radius in a leaf, without cutting the leaf.

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