CURRENT TECHNIQUES FOR THE EVALUATION OF CROSS-SECTIONAL AREA IN RAT TAIL TENDONS GENERATE SIGNIFICANT ERRORS

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INTRODUCTION

Rat tail tendons (RTTs) are commonly used tissues in biomechanical and mechanobiological studies. In order to accurately determine the stresses in these tissues, a precise evaluation of their cross-sectional area is required. Errors associated with the evaluation of cross-sectional area, and therefore stress, add to the large dispersion of data often observed with biological tissues caused by inter- and intra-individual parameters. Moreover, these errors may have implications beyond the evaluation of mechanical properties. For example, they may affect the behaviour of dead RTTs under fatigue testing or live RTTs under mechanobiological stimulation through the application of cyclic stresses.

Many research groups have discussed and improved cross-section evaluation techniques for ligament and tendon specimens. They have investigated optical, ultrasound and mechanical techniques. However, and likely because manipulating RTTs can be tedious, the cross-sectional area of these tissues is usually evaluated using low precision techniques. In most cases, RTTs are observed under light microscopy and their projections are measured using a micrometric ruler. Three techniques are then used to evaluate cross-sectional area from these measurements:

- **Single measurement circle:** The specimen's cross-sectional profile is modeled as a circle. The diameter of the circle is evaluated by one measurement of the specimen projection. The measured diameter varies with the orientation of the profile projection. (Fig. 1.A)
- **Multiple measurement circle:** The specimen's cross-sectional profile is modeled as a circle. Several measurements of the specimen projection at different rotation angles are averaged to a mean diameter. (Fig. 1.B)
- **Two degrees of freedom ellipse:** The specimen is modeled as an ellipse. Two specimen projections at 90° rotation are measured in order to get the major and minor ellipse diameters. These diameters vary with the orientation of the profile projections (Fig. 1.C)

The actual degree of error generated with these currently used methods is however unknown. Therefore, researchers cannot determine whether or not these methods are appropriate for their applications.

We have hypothesized that the errors generated are non-negligible and that an instrument could be developed to evaluate RTT cross-sectional area more accurately. In order to investigate the risks of error, we performed a theoretical comparative analysis where we evaluated the cross-sectional area of five profiles with five optical techniques. These were the three previous techniques as well as the two following techniques that were developed for primary breakdown optimizers in sawmills and ligament biomechanics, respectively:

- **Three degrees of freedom ellipse:** The specimen is modeled as an ellipse. Three specimen projections at 60° rotation are measured. Specific equations allow calculation of the major and minor ellipse diameters [1] (Fig. 1.D). For a detailed procedure, please refer to Mongeau *et al* [1]. The advantage of this method is that it optimizes the ellipse major and minor axis direction at the same time as the major and minor diameter length. The three degrees of freedom ellipses should therefore be more accurate than two degrees of freedom ellipses [1].
- **Profile reconstruction algorithm:** The specimen's cross-sectional area is modeled as a polygon delimited by the projections of the specimen measured at $\Delta\theta$ increments between 0 and 180° (Fig. 1.E). For a detailed procedure, please refer to Langelier *et al* [2] and Lee and Woo [3]. This method, an improvement over previous ones, does not fit a predefined model but instead constructs a polygon that tightly wraps the specimen profile. The profile reconstruction algorithm always overestimates the specimen's cross-sectional area since the profile is inscribed inside the polygon. The challenge with this algorithm is to measure the specimen profiles and locate them accurately within a given reference frame.

We concluded from this analysis that current techniques may generate significant errors. As well, highly accurate results are obtained for convex profiles using the reconstruction algorithm.

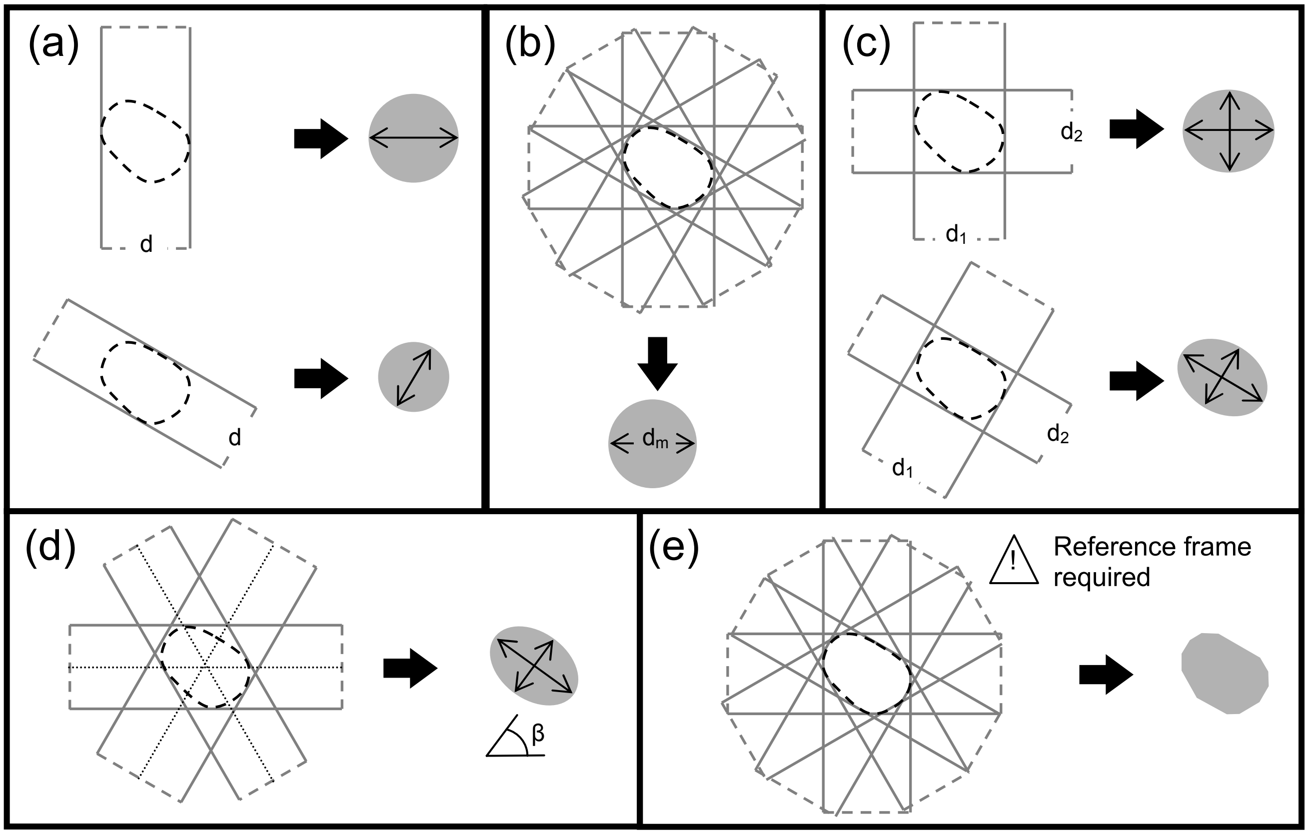


Figure 1: Section evaluation techniques compared in the theoretical analysis. (a) Single measurement circle; (b) Multiple measurement circle; (c) Two degrees of freedom ellipse; (d) Three degrees of freedom ellipse; (e) Profile reconstruction algorithm.

Based on these results, we designed an optic micrometer for use with the profile reconstruction algorithm. Using this system, we evaluated the cross-sectional area of a 0.5mm lead and a 1.5mm hex key. The errors on the measurement were all under 2%.

MATERIALS AND METHODS

Theoretical analysis

To evaluate the errors associated with each method, the five techniques for evaluating cross-sectional area were compared theoretically. These were: Single measurement circle; Multiple measurement circle where projection capturing was executed at a rotation angle increment $\Delta\theta = 10°$; Two degrees of freedom ellipse; Three degrees of freedom ellipse; and Profile reconstruction algorithm where projection capturing was executed at a rotation angle increment $\Delta\theta = 10°$ (Fig. 1). Different shapes were generated with drawing software (Fig. 2). The bicolour images were imported in Matlab and converted into matrices composed of zeros and ones. X-Y coordinates of zeros were recorded since they allow characterization of the shape profiles. For each predefined direction (θ), profile projections were evaluated as the distance between the two most distant zeros (Fig. 2). The estimated cross-sectional areas (A_e) were determined using the five techniques. The real cross-sectional areas (A_r) were determined using the Matlab function *polyarea.* Since for most shapes, the initial orientation affects the accuracy of the cross-sectional area estimation, several estimates of the cross-sectional area were calculated using various initial orientations. Error was calculated as:

$$\varepsilon = \frac{A_e - A_r}{A_r} \times 100\% \qquad (1)$$

Five shapes were tested: a circle, a large aspect ratio ellipse, a small aspect ratio ellipse and two tendon cross-sectional profiles drawn from images of published articles [4-5].

Apparatus

The designed optic micrometer (Fig. 3) is to be used

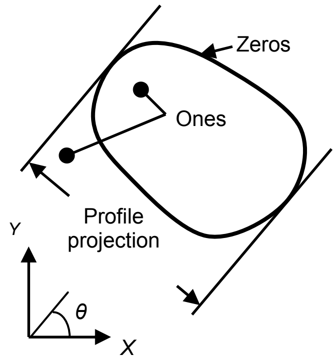


Figure 2: A bicolour shape is generated and converted into a matrix composed of zeros and ones. For each predefined direction (θ), the profile projection is evaluated as the distance between the two most distant zeros.

with a stereomicroscope equipped with a digital camera (not shown). To maintain hydration, the specimen is immersed in a measuring compartment filled with saline solution. It is attached to two rotating shafts which are guided by two long and tight fitting cylindrical openings on the right and left sides of the measuring compartment. High precision machining of these openings is important because axial alignment of the shafts is crucial. It is important to minimize specimen movement in the y axis to stay in the focused zone determined by the microscope's depth of field. Outside this zone, captured images would be fuzzy and refocusing would shift the local reference frame (i.e. the distance between the specimen and the picture upper border) used in the profile reconstruction algorithm.

Cross-sectional area evaluation

For each magnification ratio, picture calibration (pixel vs µm) was accomplished using a micrometric ruler. Pictures of the specimens were analyzed with Vision assistant (Version 7.1 National Instrument, Austin, Texas, USA). Upper and lower specimen edges, found by contrast, were located within a local reference frame. Then, a Matlab routine executed the profile reconstruction algorithm [2] for data obtained at six predefined sites along the specimen to obtain six cross-sectional area evaluations for each series of pictures.

Validation of the optic micrometer

Experimental validation of the designed optic micrometer was performed using a 0.5 mm lead, a 1.5mm hex key as well as frozen-thawed tendons and freshly isolated tendons. The profiles of these samples were reconstructed with the profile reconstruction algorithm where projection capturing was executed at

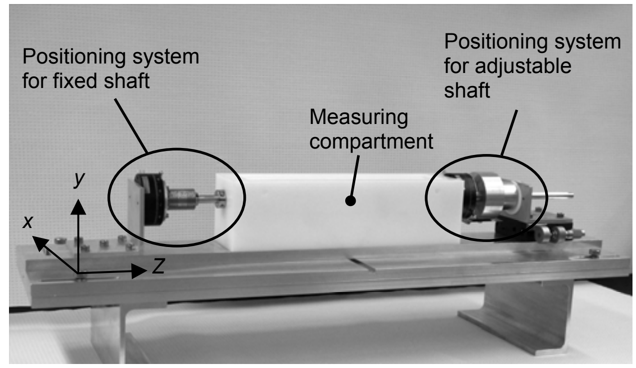


Figure 3: Global view of the optic micrometer.

a rotation angle increment $\Delta\theta = 5°$ for the lead and $\Delta\theta = 10°$ for the key and tendons. The reconstructed profiles of the lead, key and tendons were used to different purposes:

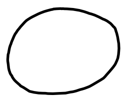
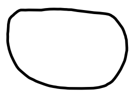
- **Profile:** We compared the estimated profile of the lead with its real profile by superimposing the outline of one reconstructed profile on a picture of the lead's extremity. We also verified that the reconstructed profiles of the hex key were hexagonal.
- **Error:** We evaluated error on the estimation of the cross-sectional area (Equation 1) for both the lead and the hex key. The real cross-sectional area of the lead was evaluated from the picture of its extremity. Using Vision assistant, we determined the lead's outline and counted the number of pixels inside. With the appropriate calibration factor, we converted the number of pixels to an area. With the hex key, we measured the distance between opposite sides with a calliper and evaluated its real cross-sectional area geometrically.
- **Tendon handling:** We tested the optic micrometer with frozen-thawed tendons as well as freshly isolated tendons. To avoid sagging, a small tension corresponding to a 3% elongation was applied to the specimens.

RESULTS

Theoretical analysis

- The single measurement circle technique always generates the largest range of errors (greatest averages and standard deviations) for ellipses and tendon shapes. The multiple measurements circle technique generates very small error ranges for each shape, but may produce a relatively important range of error for the five shapes combined. The two degrees of freedom ellipse technique always produces average errors that are smaller than those obtained with the multiple measurement circle technique, but with much larger ranges. The three degrees of freedom technique

Table 1: Theoretical analysis of errors generated with the cross-sectional area evaluation techniques for the two RTT profiles. A) Single measurement circle; B) Multiple measurement circle; C) Two degrees of freedom ellipse; D) Three degrees of freedom ellipse; E) Profile reconstruction algorithm.

Tested profiles			**A**	**B**	**C**	**D**	**E**
RTT 1		Max $\|\varepsilon\|$	35.08	4.18	6.97	2.82	0.61
		$\bar{\mathcal{E}} \pm \sigma(\varepsilon)$	5.22 ± 21.04	4.15 ± 0.02	3.11 ± 2.97	-1.88 ± 0.78	0.60 ± 0.01
RTT 2		Max $\|\varepsilon\|$	47.56	10.09	19.25	11.93	1.39
		$\bar{\mathcal{E}} \pm \sigma(\varepsilon)$	11.97 ± 28.23	10.04 ± 0.03	8.38 ± 9.14	-0.45 ± 7.41	1.18 ± 0.14

seems to be a good method for shapes that are similar to ellipses, but errors may be more important with other shapes. Finally, the profile reconstruction algorithm gives the best results with a very narrow range of errors, under 2%.

Results from the theoretical analysis applied to RTTs are presented in Table 1. Results for the other shapes are not shown.

Validation of the optic micrometer

- **Profile:** The reconstructed profile of the lead closely superimposes on the outline and the reconstructed profiles of the hex key are hexagonal (Fig. 4).
- **Error:** For the lead, the error on the cross-sectional area evaluation is 0.8%. For the hex key, the error varies from -1.8 to 1.0% for the six predefined sites at a 0.4mm interval along the key.
- **Tendon handling:** Installing a tendon and taking pictures takes an average of 30 minutes, but becomes more rapid with training.

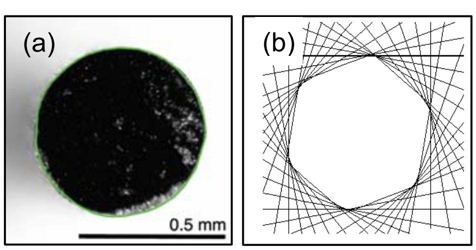


Figure 4: (a) Reconstructed profile of the lead superimposed on a picture of its extremity; (b) Reconstructed profile of the hex key. Lines represent the upper and lower edges recorded on the series of pictures.

DISCUSSION

Taken together, our results demonstrate that current techniques for the evaluation of the cross-sectional area of RTTs generate significant errors, whereas use of the designed optic micrometer along with the profile reconstruction algorithm allows evaluation of the cross-sectional area of RTTs with high accuracy.

Since tissue cross-sectional area is essential for the normalization of stress-strain curves, improvement of this measurement could have a positive impact in biomechanical and mechanobiological studies using RTTs by decreasing inter-group data variability and thus facilitating the discrimination between different experimental groups. Our future work will explore this question.

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