

Application of Digital Image Correlation to the Local Strain Analysis of Mouse Aortas: Novel Method to Create Speckle Pattern

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ABSTRACT

A novel method utilizing colloidal gold particles to create speckle patterns on mouse aortas for digital image correlation (DIC) studies is presented. The colloidal gold particles form random, stable, high contrast, variably sized speckles suitable for DIC analysis and can be used to measure local strains during mechanical failure testing.

Keywords: Digital image correlation, Speckle pattern, Colloidal gold particles, Strain field measurement, Mouse aorta

INTRODUCTION

Accurate measurement of the complex strain fields of biological samples is essential to the field of experimental biomechanics. Among the different techniques for strain measurement, digital image correlation (DIC) provides a non-contact optical method that enables direct measurements of the deformation and strain of materials without affecting their mechanical properties. This method is based on optically tracking the displacement of speckle patterns created on the sample surface. There are several advantages to using DIC to study vascular samples since it can provide local, rather than global, deformations and it is suitable for large deformation measurements, typical of soft tissues taken to failure ^{[1][2]}.

To optimize DIC, several requirements should be met for speckle patterning: 1) randomness, 2) high contrast, 3) appropriate size of speckle in the field of view (3-5 pixels), and 4) firm attachment of speckles to the specimen during deformation. Previous DIC studies of soft tissues created speckle patterns using an airbrush to spray dye or paint or coating the sample with toner powder. However, biological samples must be partially dehydrated before applying paint, affecting their mechanical properties, and toner powder is too hydrophobic to adhere well when submerged in aqueous solutions during mechanical testing. In addition, it is difficult to evenly distribute paint or toner powder on the surface of a hydrated biological specimen ^[2]. Therefore, a novel method exploiting colloidal gold particles to form a speckle pattern on mouse aortas is proposed in this work. Due to their unique surface chemistry including coordinate covalent and hydrophobic interactions with proteins, chemical inertness and stability in aqueous environments, colloidal gold particles are a promising new candidate for speckle patterning for DIC strain measurements of mouse aortas and similar small biological specimens ^[3].

MATERIALS AND METHODS

Colloidal gold particles were synthesized by reducing chloroauric acid (HAuCl₄) in an ascorbic acid solution. In this method, 125 μ l of 0.1 M chloroauric acid was added into 10ml of 0.3M ascorbic acid solution with vigorous stirring for 15 min. The solution was then incubated at room temperature for at least 96 h. Gold nanoparticles aggregated and eventually the size of aggregated gold particles was tuned in the range of 1-10 μ m. The acidic supernatant was separated from the suspension, and the remaining precipitated gold particles were resuspended in phosphate-buffered saline (PBS) solution. The mouse aorta samples were then soaked in the colloidal gold particle suspension. As a result of coordinate covalent and hydrophobic interactions between gold particles and arterial wall proteins, spontaneous adsorption of gold particles onto the tissue surface occurred to form a random speckle pattern. The patterned sample was then mounted to a Bose mechanical test bed to conduct uniaxial tensile ring tests with a CCD camera capturing images of the patterned surface throughout the test. Image acquisition

and DIC settings include a 2 MP Point Grey camera with 25 mm macro lens and a 35 x 35 subset for VIC-2D. For inherent system error analysis ten consecutive images of a stationary (prior to stretching) speckled mouse aorta were captured and analyzed in VIC-2D. The image acquisition interval for all tests was specified as 200ms, and a series of images were captured consecutively throughout stretching until the sample failed. Images were imported into VIC-2D software to obtain the strain field information. DIC image analysis was performed in a stepwise manner at approximately 20 images per step. The final image in each group was set as the new reference image for each subsequent step.

ANALYSIS

Figure 1 shows a random speckle pattern created on a mouse aortic ring specimen. The size of a typical random speckle covers 3-5 pixels in each direction, which is suitable for optimum image tracking. In Figure 2, the intensity histograms of patterned specimens had greyscale values in the range 60-255, indicating a sufficient level of contrast between the mouse aorta and the speckle pattern for reliable image matching by DIC. Figure 3 demonstrates that after the sample had been submerged in PBS for 1 hour, the random speckle pattern was still stable in an aqueous environment.

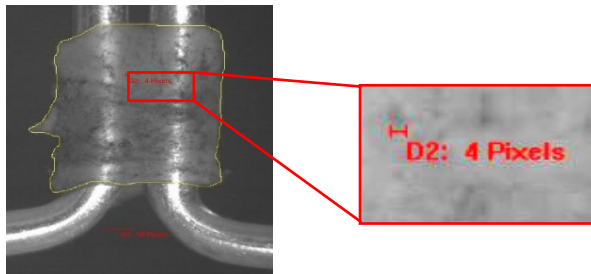


Fig. 1 Patterned mouse aorta mounted on cannulas on Bose mechanical test bed

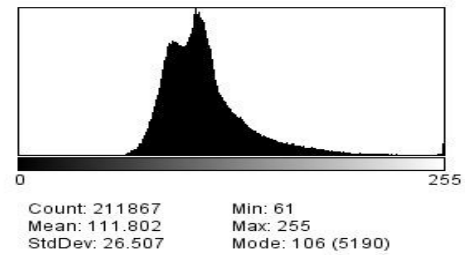


Fig.2 Intensity histogram analysis of region of interest illustrated in Figure 1

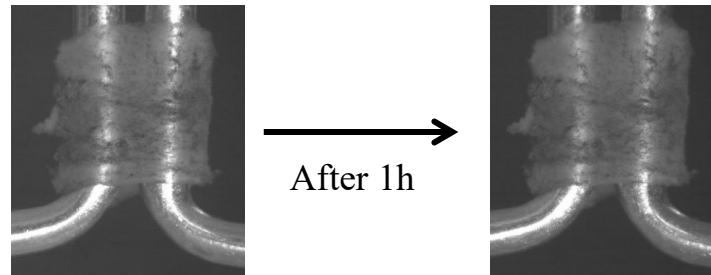


Fig. 3 Patterned mouse aorta submerged in PBS for 1 hour

For system error analysis, the corresponding pseudo-strain measurements (mean, median and standard deviation) for ϵ_{xx} , ϵ_{yy} and ϵ_{xy} of a static specimen are summarized in Table 1.

Table 1. System error analysis: pseudo-strain data in different directions

	ϵ_{xx}	ϵ_{yy}	ϵ_{xy}
Mean	0.00228	-0.00065	-0.00435
Median	-0.00357	0.0043	-0.00643
Standard deviation	0.01134	0.01254	0.00666

In Figure 4, strain measurement of the speckle patterned mouse aorta was compared over the whole specimen (a) and in the central region (b) in the y-direction during a uniaxial mechanical tensile test. In Figure 4a, the strain field was highly heterogeneous over the whole specimen. Strains around the peripheral regions of the sample were significantly higher than in

the central region due to bending and friction at the mounting arms. Local strains of the ring specimen in uniaxial tension were more homogeneous in the central region, as illustrated in Figure 4b, although there were still some edge effects due to the small size of the tissue sample relative to the mounting arms.

In Figure 5, the local strain fields of a patterned mouse aorta correspond to different incremental stages during a uniaxial mechanical test-to-failure. The first row of images (a.-c.) illustrates the early stages of extension testing with small displacements and progressively increasing local strain values corresponding to mounting arm displacements in the y-direction. More heterogeneity in the strain data can be observed in the second row of images (d.-f.) and the greatest increase in strain was concentrated in the center of the sample. These data point to the difficulty of achieving uniform loading in uniaxial tensile tests where the vessel diameter is only 1 mm. In the last row of images, when the sample was stretched to its failure point, there was little variation in strain on the sample with increasing displacement of the mounting arms since, at this point, the load-bearing components of the arterial wall have mostly failed. Likewise, pattern tracking using VIC-2D software begins to fail, as indicated by the areas of missing data. Based on these results, it is feasible to obtain the local strain field of mouse aortas using our approach during uniaxial mechanical extension up to the point of material failure.

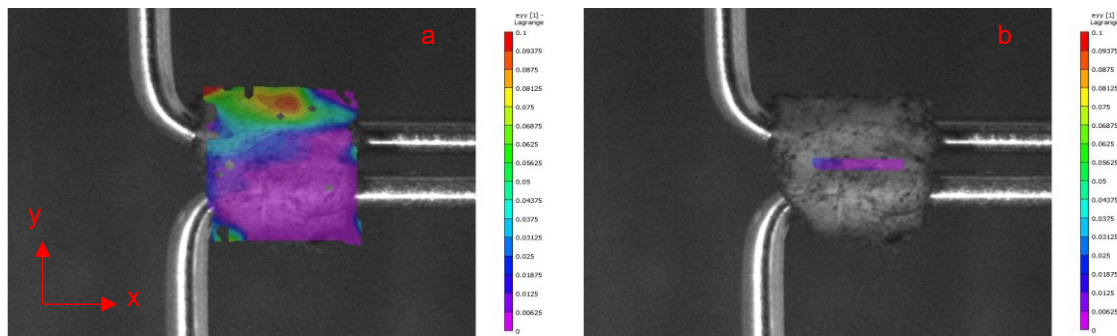
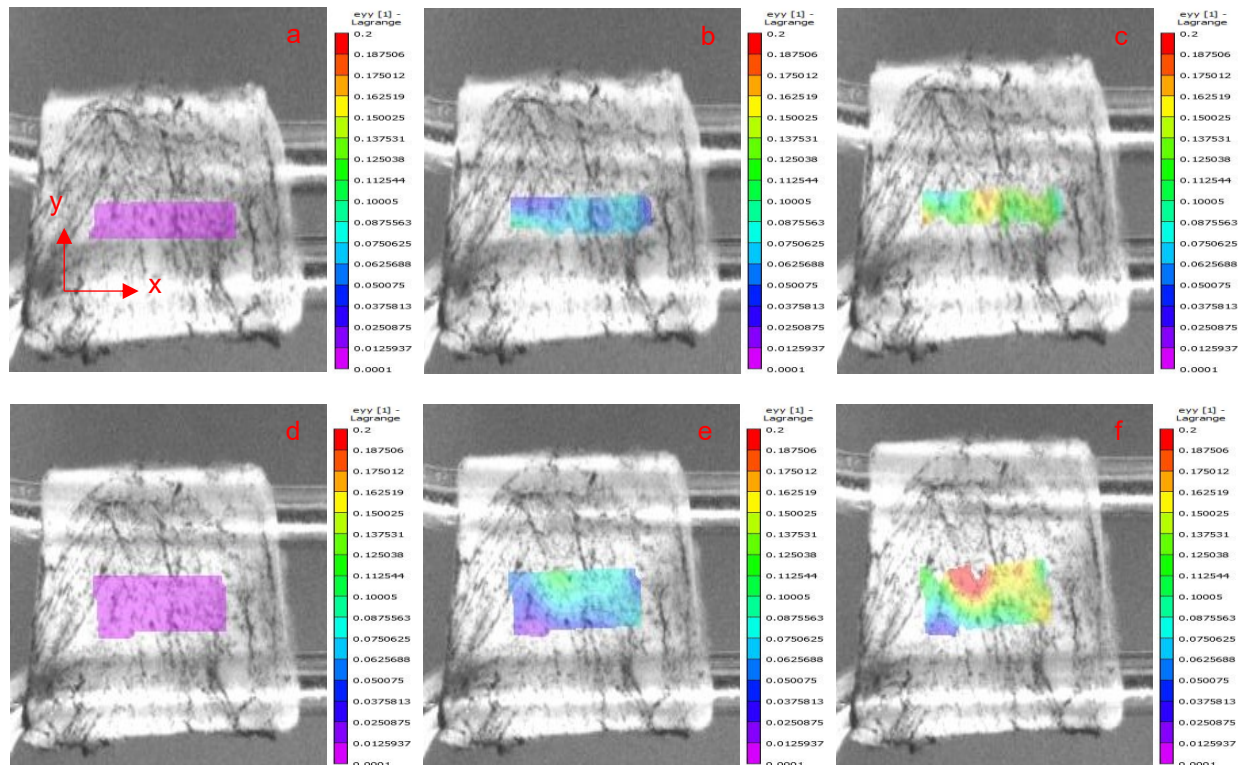


Fig. 4 Strain analysis of a patterned mouse aorta in y-direction a. over the whole specimen; b. in the central region



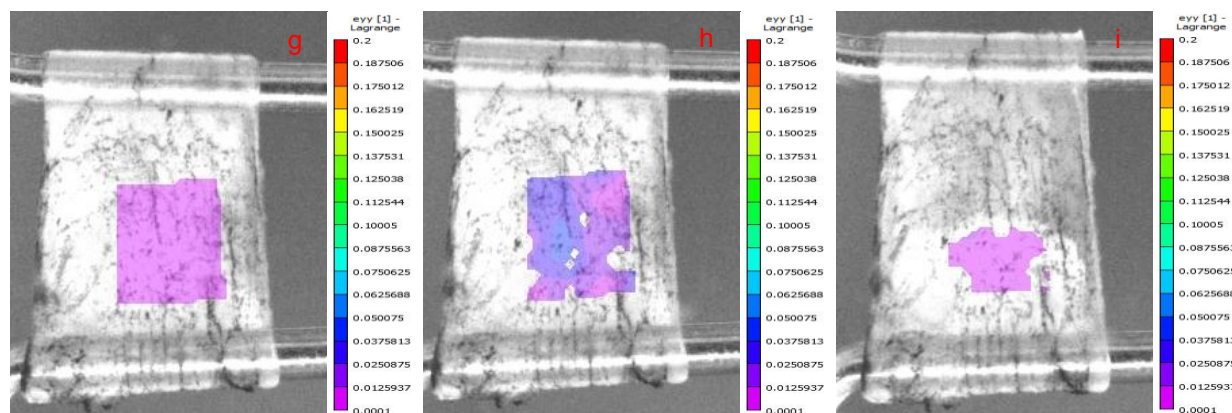


Fig. 5 Local strain measurements of a speckle patterned mouse aorta for progressive displacement stages during uniaxial pull-to-failure testing: a.-c. examples of the local strain field for low displacement ranges, arranged in consecutive order from left-to-right; d.-f. examples of local strain field for the medium displacement ranges, arranged in a consecutive order from left-to-right; g.-i. examples of the local strain field close to material failure. Note that the leftmost image in each series serves as the reference image for DIC speckle pattern tracking in that series.

CONCLUSION

A novel method utilizing colloidal gold particles to create a speckle pattern on mouse aortas shows promising results compared to previous methods. The colloidal gold particles form random, stable, high contrast, appropriately sized speckles suitable for DIC analysis during tensile failure tests of fully hydrated mouse aortas. During the image analysis process, unique patterns in each subset region were correlated image-by-image using an optimization algorithm to map the displacement of the subset region and to calculate the strain field. DIC was successfully applied in a stepwise fashion to accommodate large distortions in the starting pattern and loss of correlation at high strain values. Based on these results, a new patterning method can be applied to the strain field measurement of samples undergoing large deformations during uniaxial tensile failure experiments.

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