# Pulsatile variation of the chick extraembryonic arterial bifurcation observed by ultrasound and optical microscope

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## 1. Introduction

During the development of the chick extraembryonic circulatory system, the hemodynamic forces acting on the blood vessels result in the local changes in wall shear stress (WSS) distribution. However the most of previous study on the extraembryonic circulation in chorioallantoic membrane (CAM) were mainly based on the static geometry of veins and arteries. This paper aims to the pulsation variation of investigate the geometrical variation of the arterial bifurcation using the chick embryo CAM as an experimental model. Using a computer based ultrasound imaging high-frequency cross-sectional system, 2-D ultrasound images were obtained and constructed a 3-D image. In addition, an optical microscope was used to obtain the images of pulsatile arterial motion in the CAM. The ultrasound and optical images of the CAM arteries were analyzed by an image-processing techniques.

## 2. Experimental Procedure

The chicken eggs were incubated in a digital incubator (RCOM PRO 50, Autoelex Co, Korea) at 37.5  $^{\circ}$ C and 60 % humidity. After 3 days of incubation, The eggs were carefully cracked and placed on a petridish. The microscope images of the

CAM arteries were obtained at the days of 11 of incubation using a digital video camera recorder (Sony HDR-XR520, Japan). Fig. 1 shows the microscope image of the chorioallantoic artery bifurcation.



Fig. 1 The chick embryo chorioalIntoic arteryat day of 11 of incubation

A computer based high-frequency ultrasound probe with a 35-MHz transducer (Capistrano Labs., San Clemente, CA, USA) was used. **Fig. 2(a)**, **2(b)**, and **2(c)** show the schematic of the experimental setup, a chick chorioallantoic artery bifurcation captured by a digital camera, and cross-sectional ultrasound images of the artery bifurcation, respectively.

## 3. Image Analysis

The vessel boundary detection based on brightness thresholding and correlation coefficient mapping were employed on 2-D cross-sectional ultrasound images. Using the analysis results, 3-D

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geometry of artery bifurcation was reconstructed. Spectral analysis based on FFT was employed on optical images to investigate arterial wall dynamics.



Fig. 2(a) A computer based ultrasound imagin g system with a 35-MHz probe



Fig. 2(b) Representative photo of the CAM arteries in chick embryo model



Fig. 2(c) The cross-sectional ultrasound images of chorioallantoic artery bifurcation

### 4. Results and Discussion

By analyzing cross-sectional B-mode ultrasound images, we obtained 3D information of arterial bifurcation geometry in chick CAM as shown in **Fig. 3.** We also measured the spatio-temporal variation of arterial movement near bifurcation during a cardiac cycle on both ultrasound images and optical microscope images. The experimental results demonstrate that the arterial wall expands asymmetrically during a cardiac cycle, and the wall movement shows the local variation near the arterial bifurcation.



Fig. 3 3-D geometry of the CAM artery bifurcation reconstructed from ultrasound images.

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