Time-lapse Optical Coherence Tomography Embryo Imaging with Minimal Disturbance

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Abstract: We present a optical coherence tomography system for minimally-disturbance imaging of porcine embryos, placed inside an incubator which ensures adequate environmental parameters including temperature, humidity, and gas ratios.

In farm animal embryo production systems that rely on *in-vitro* fertilization (IVF), the embryo is transferred into a recipient animal once it reaches the blastocyst stage, but the eligibility for transfer requires a morphological assessment of each candidate embryo [1]. While observation of embryo morphology is relatively straight-forward with conventional microscopy techniques in human IVF, the same procedure cannot be applied to cattle (and other mammalian) embryos, due to the accumulation of lipid droplets in their cytoplasm [2].

Additionally, to support the morphological assessment of embryos whilst providing a better prediction of viability, time-lapse imaging systems are widely utilized in human IVF [3]. In these systems, full investigation requires embryo culture and observation over a long period of time, which limits the scope of their application; moreover, due to the limitations in terms of contrast explained in the previous paragraph, cattle embryos are not as easily imaged by this technique because of their lipid content.

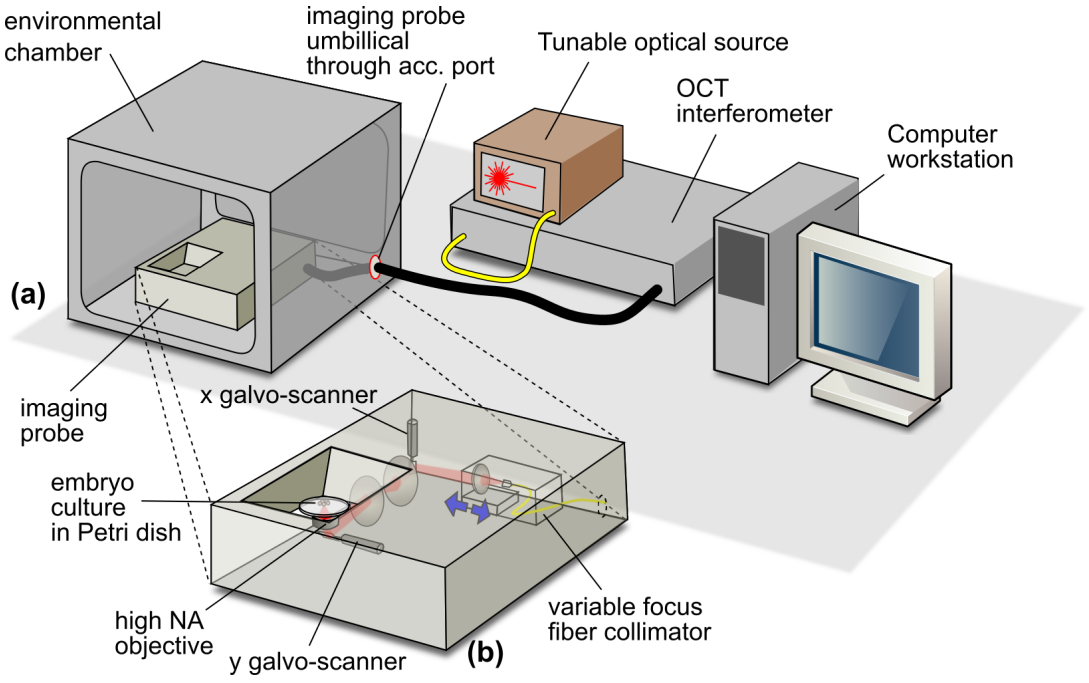

Fig. 1. (a) Schematic representation of the OCT system presented in this communication, comprising OCT interferometer, tunable optical source, and external imaging probe which is installed inside the environmental chamber; (b) close-up of the imaging probe, detailing where the imaging dish containing the embryos is mounted.

To improve contrast, and fully characterize the embryo's morphology in three dimensions, other imaging modalities have been employed, such as optical coherence tomography (OCT) [4]. Since its debut in 1991 [5] , OCT

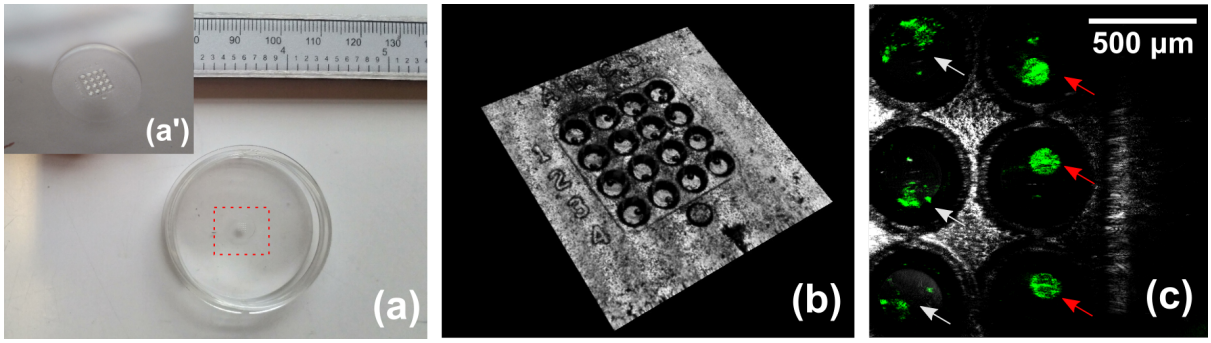

Fig. 2. (a) photograph of the multi-well Primo Vision dish containing the microbeads, inset (a') is a close-up of the microwell array in the center of the dish; (b) 3-D render showing the microwell imaging dish and the microbeads (taken with a separate, large lateral range SS-OCT system), approximately $4 \times 4\,\text{mm}^2$ lateral scan area; (c) composite *en-face* image acquired with the environmental chamber imaging probe, gray color corresponding to the depth where the outer structure of the microwells is located, and the green color corresponding to the depth plane where the 100 µm-diameter microbeads emulating the embryos are located. Red-arrowed microbeads were in the focal plane, whereas the white-arrowed ones were not.

imaging has been widely used in the medical and biomedical fields. This technique [6] relies on the principle of low-coherence interferometry to depth profile scattering samples, with a resolution of tens of microns and over a range of a few millimeters (sample-dependent). More recently, novel advances in processing the OCT data, coupled with large increases in imaging throughput, allowed the field of OCT angiography to gain increasing importance; this functionality extension of OCT imaging allows the identification of moving scatterers in OCT volumes, which can be linked to metabolic activity and cell division.

The aforementioned study by Caujolle *et al* [4] presented some limitations, though, namely the fact that it was carried out in a bench-top OCT imaging system requiring the embryos to be removed from their optimum environmental parameters (temperature, humidity, gases) for each imaging session taking place. This has the obvious drawback of adversely affecting their development process [7], particularly since embryo development and viability are heavily dependent on environmental parameters, particularly temperature and CO_2 concentration.

In this communication, we introduce a swept-source based OCT imaging system comprising a high resolution imaging probe designed to be installed inside an IVF incubator, and an OCT interferometer providing the reference path. This system is illuminated by a commercial swept source (Axsun) operating at 1060 nm, with a tuning bandwidth of 100 nm and a sweep rate of 100 kHz, ensuring an axial resolution of $\sim 10\,\mu\text{m}$. The full system is schematically represented in Fig. 1. The OCT data is processed with the Complex Master/Slave OCT (CMS-OCT) procedure [8], allowing direct *en-face* image rendering.

The imaging probe (Fig. 1 (b)) is encased in a box following the IPX67 standard, ensuring full protection to all the optical and opto-mechanical components from the high humidity inside the environmental chamber. To guarantee a high lateral resolution of $2-3\,\mu\text{m}$, a short-f objective ($f = 10\,\text{mm}$) was chosen, and to minimize beam-walkoff a split-scanner telecentric configuration was adopted. Moreover, for optimum positioning of the narrow confocal gate, a custom-made, computer-controlled variable focus fiber collimator is also present.

The samples are placed in a specially-devised imaging dish, containing 9 micro-wells (Vitrolife, Primo Vision culture dish, model 16604), and imaged from underneath the dish (Fig. 1 (b)). A color photograph of this imaging dish is shown in Fig. 2 (a). Each microwell is approximately 500 µm in diameter, and 300 µm deep.

For a first demonstration of the system functionality, we have placed plastic microbeads (provided by *Research Instruments*, and approximately 100 µm in diameter) within each microwell (1 per microwell). In terms of physical footprint, the beads have a size that compares favorably with a pig oocyte or a pig embryo of 1 to 4 days of age. A pig blastocyst (day 5) may have this size but will likely become bigger than the bead by the end of day 5.

In Fig. 2 (b), a 3-D render of an OCT volume covering the entire center of the microwell dish is shown. This volume was obtained with a separate OCT system having the interface optics above the dish; the system has been described elsewhere [9], and allows large ($> 10\,\text{mm}$) lateral imaging.

Preliminary results obtained with the imaging probe are shown in Fig. 2 (c), where a composite image is presented, with the outer structure surrounding the microwells represented in gray color and the microbeads in green. The microbeads in the left column appear distorted, as they were not exactly on the same depth plane as the ones in the center column. More results will be communicated at the meeting, namely those involving real embryos, and with the probe operating inside the environmental chamber under the appropriate parameters.

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