University of Kent

Kent Academic Repository

Marques, M.J., Serrano-Albal, María, Camard, Julien, Silvestri, Giuseppe, Canedo-Ribeiro Carla, Robinson, Gary K., Griffin, Darren K., Bradu, Adrian and Podoleanu, Adrian G.H. (2021) *Time-lapse optical coherence tomography embryo imaging with minimal disturbance.* In: Proceedings Volume 11924, Optical Coherence Imaging Techniques and Imaging in Scattering Media IV. (1192). SPIE, Romania

Downloaded from
https://kar.kent.ac.uk/109649/ The University of Kent's Academic Repository KAR

The version of record is available from
https://doi.org/10.1117/12.2616149

This document version
Publisher pdf

DOI for this version

Licence for this version
UNSPECIFIED

Additional information

Versions of research works

Versions of Record
If this version is the version of record, it is the same as the published version available on the publisher's web site. Cite as the published version.

Author Accepted Manuscripts
If this document is identified as the Author Accepted Manuscript it is the version after peer review but before type setting, copy editing or publisher branding. Cite as Surname, Initial. (Year) 'Title of article'. To be published in ***Title of Journal*** , Volume and issue numbers [peer-reviewed accepted version]. Available at: DOI or URL (Accessed: date).

Enquiries
If you have questions about this document contact ResearchSupport@kent.ac.uk. Please include the URL of the record in KAR. If you believe that your, or a third party's rights have been compromised through this document please see our Take Down policy (available from https://www.kent.ac.uk/guides/kar-the-kent-academic-repository#policies).

Time-lapse Optical Coherence Tomography Embryo Imaging with Minimal Disturbance

Manuel J. Marques[1,*], María Serrano-Albal[2], Julien Camard[1], Giuseppe Silvestri[2], Carla Canedo-Ribeiro[2], Gary Robinson[2], Darren Griffin[2], Adrian Bradu[1], and Adrian Podoleanu[1]

[1] *Applied Optics Group, School of Physical Sciences, Division of Natural Sciences, University of Kent, Canterbury CT2 7NH, United Kingdom.*

[2] *School of Biosciences, Division of Natural Sciences, University of Kent, Canterbury CT2 7NJ, United Kingdom.*

* M.J.Marques@kent.ac.uk

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. © 2021 The Author(s)

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.

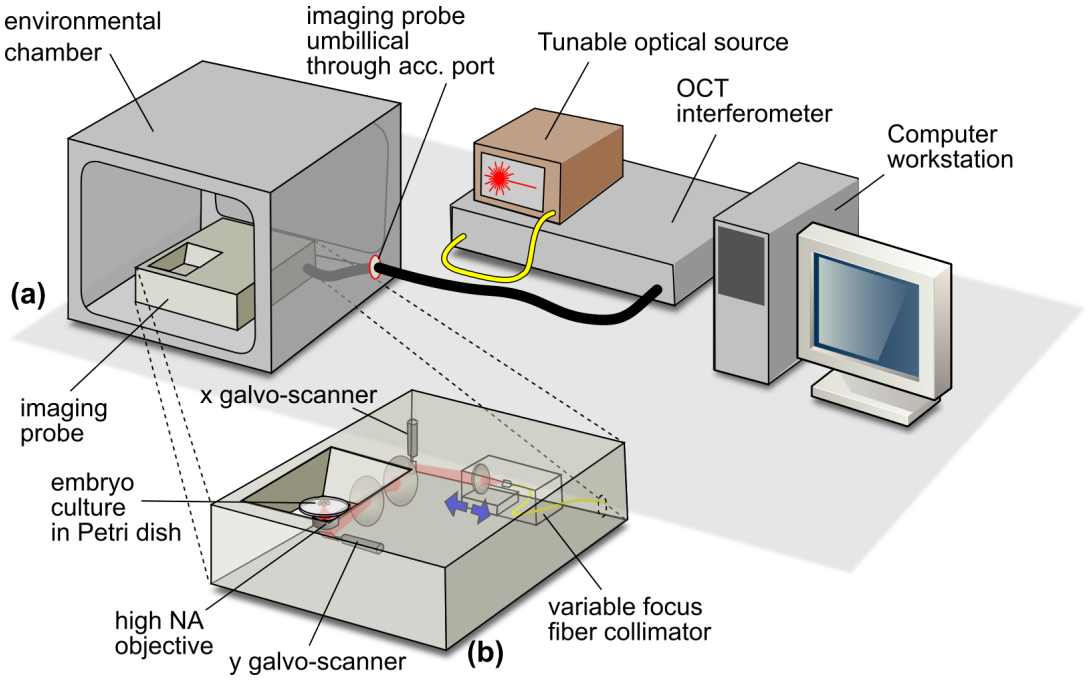


Fig. 1. (a) Schematic representation of the OCT system presented in this communication, comprising OCT interferometer, tunable optical source, and external imaging probe which is installed inside the environmental chamber; (b) close-up of the imaging probe, detailing where the imaging dish containing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

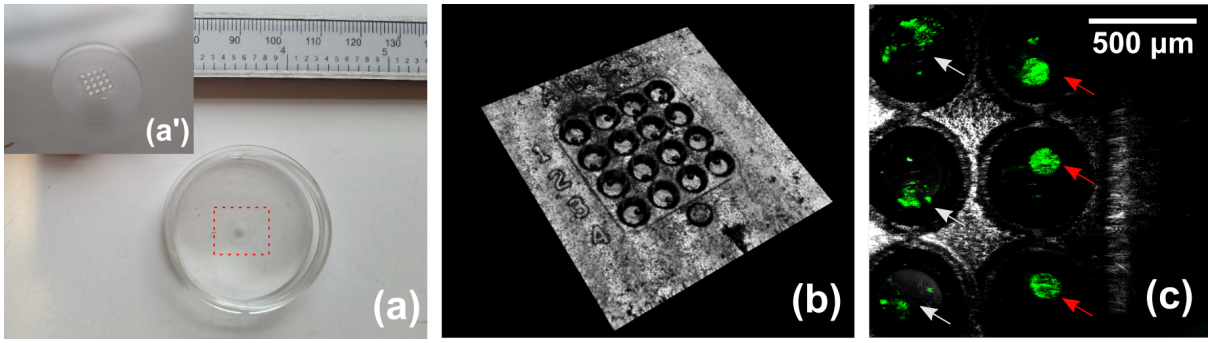


Fig. 2. (a) photograph of the multi-well Primo Vision dish containing the microbeads, inset (a') is a close-up of the microwell array in the center of the dish; (b) 3-D render showing the microwell imaging dish and the microbeads (taken with a separate, large lateral range SS-OCT system), approximately $4 \times 4\,\text{mm}^2$ 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.

Acknowledgements

The authors acknowledge the support from the Biotechnology and Biomedical Sciences Research Council (*5DHiResE* project, grant number BB/S016643/1). A. Podoleanu also acknowledges support from the National Institute for Health Research (NIHR) Biomedical Research Centre at the UCL Institute of Ophthalmology, University College London, the Moorfields Eye Hospital NHS Foundation Trust, and the Royal Society Wolfson research merit award.

References

1. C. Pribenszky, A.-M. Nilselid, and M. Montag, "Time-lapse culture with morphokinetic embryo selection improves pregnancy and live birth chances and reduces early pregnancy loss: a meta-analysis," Reproductive BioMedicine Online **35**, 511–520 (2017).
2. A. Van Soom, B. Mateusen, J. Leroy, and A. de Kruif, "Assessment of mammalian embryo quality: what can we learn from embryo morphology?" Reproductive BioMedicine Online **7**, 664–670 (2003).
3. T. Somfai, Y. Inaba, Y. Aikawa, M. Ohtake, S. Kobayashi, K. Konishi, and K. Imai, "Relationship Between the Length of Cell Cycles, Cleavage Pattern and Developmental Competence in Bovine Embryos Generated by In Vitro Fertilization or Parthenogenesis," J. Reproduction Dev. pp. 0912210236–0912210236 (2009). Publisher: The Society for Reproduction and Development.
4. S. Caujolle, R. Cernat, G. Silvestri, M. J. Marques, A. Bradu, T. Feuchter, G. Robinson, D. K. Griffin, and A. Podoleanu, "Speckle variance OCT for depth resolved assessment of the viability of bovine embryos," Biomed. Opt. Express **8**, 5139 (2017).
5. D. Huang, E. A. Swanson, C. P. Lin, J. S. Schuman, W. G. Stinson, W. Chang, M. R. Hee, T. Flotte, K. Gregory, C. A. Puliafito, and A. Et, "Optical coherence tomography," Science **254**, 1178–1181 (1991). Publisher: American Association for the Advancement of Science Section: Reports.
6. A. G. Podoleanu, "Optical coherence tomography," J. Microsc. **247**, 209–219 (2012).
7. J. Q. Zhang, X. L. Li, Y. Peng, X. Guo, B. C. Heng, and G. Q. Tong, "Reduction in exposure of human embryos outside the incubator enhances embryo quality and blastulation rate," Reproductive BioMedicine Online **20**, 510–515 (2010).
8. S. Rivet, M. Maria, A. Bradu, T. Feuchter, L. Leick, and A. Podoleanu, "Complex master slave interferometry," Opt. Express **24**, 2885 (2016).
9. M. J. Marques, S. Rivet, A. Bradu, and A. Podoleanu, "Complex master-slave for long axial range swept-source optical coherence tomography," OSA Continuum **1**, 1251 (2018).