Assessing embryo quality with digital holographic microscopy

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Abstract. A low-powered, non-invasive digital holographic microscopic imaging technique for embryo quality assessment is presented. Two groups of 2 cell-stage embryos cultured in media of different lipid concentrations have been characterized with digital holographic microscopy for lipid aggregation. The study suggests refractive index measurements are reflective of the lipid and dry mass content in embryos thus making DHM a prospective label-free diagnostic tool for quality assessments in assisted reproduction.

1 Introduction

In vitro fertilization (IVF) success – birth of a baby - relies upon the selection of high-quality embryos. Current assessment procedures are performed either using invasive biopsy or subjective visual inspection or other imaging techniques which track dynamics and metabolism within the embryo [1]. However, little attention has been paid to the selection of quality embryos based on physical parameters, including the dynamic changes in the refractive properties during preimplantation embryo development. Here, we report on the use of low-power digital holographic microscopy (DHM) to study the physical changes in the lipid concentration and dry mass density of live murine embryos. We show that morphological information obtained via DHM is reflective of intracellular lipid aggregation and dry mass content: embryos with higher lipid content have a higher refractive index as well as greater dry mass density compared to embryos with low lipid content. The significance of this work lies in the capacity to rapidly and non-invasively acquire intrinsic phase change information and by extension lipid content from a developing cell.

2 Results

2.1. Materials and methods

Cryo-preserved 2 cell-stage mouse embryos were thawed and cultured in Research Cleave medium supplemented either without (low lipid) or with (high lipid) 10% fetal bovine serum. 2 cell-stage embryos imaged at 6 hours post thawing in Research Wash medium that has a refractive index of 1.335 and overlaid with paraffin oil. The digital holographic imaging system used is based on a Mach-Zehnder interferometer. Light from a λ=532 nm laser is split into an object and a reference beam path. The object beam illuminates the sample before recombining with the reference beam to form a hologram. Excitation power at the sample plane was 25 µW. The sample stage was heated to 37°C to maintain a constant incubation temperature. Full and detailed information on the imaging system and sample preparation procedures can be found in Ref [2,3]. The phase map is retrieved from the hologram by applying a reconstruction algorithm as detailed in Ref [4]. The reconstructed phase is a phase difference Δφ and it relates the excitation wavelength (λ), sample thickness (h) and the refractive index of the sample (n_s) surrounded by a fluid medium of index n_m by

\[ \Delta \phi = \frac{2\pi}{\lambda} (n_s - n_m) h \] (1)

2.2 Refractive index (RI) of live embryos

![Image](https://example.com/figure1.png)

Fig. 1. Phase information and refractive index maps for 2-cell stage embryos cultured in low- and high-lipid media. (a,b) Bright-field images of 2-cell stage embryos (c,d) and (e,f) represent the reconstructed 2D phase and refractive index profiles respectively. Scale bar: 30 µm.

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Figure 1 shows the phase and refractive index of 2 cell-stage embryos cultured in low and high lipid media. By visual observation of the bright field images (a,b), one may conclude that both embryos are morphologically the same. However, from the extracted phase (c,d) and RI (e,f) maps, there is a considerable difference in the phase and RI between the low and high lipid groups. This confirms that DHM is capable of detecting small changes in lipid abundance within the embryo that are not visible to the naked eye.

Figure 2 shows the mean distribution of RI in 2 cell embryos across the two lipid groups. Each black spot represents the RI for a single 2 cell-stage embryo. The mean RI values are indicated with the crossed circles while the median for each group is indicated by the solid lines within the box. Each box represents values within the 25th and 75th percentile. This shows a general increase in the RI of embryos cultured in high lipid medium. The variation of RI across other stages of development are presented in Ref [2].

Fig. 2. Mean refractive index distribution for 2-cell embryos cultured in low- and high- lipid media.

2.3 Estimation of dry mass of embryos

Dry mass measurements are critical in understanding cell size and growth regulation [5]. It is the ratio of the dry solid to the total volume of the wet sample. The dry mass of a cell is dominated by its protein content with a significant contribution from its lipid composition. The dry mass density C[g/dL] of cells is related to the RI by the expression:

\[
n_{s}(r, z) = n_{m}[\alpha(\lambda)C(r, z) + 1]
\]

where \(\alpha(\lambda)[dL/g]\) is the specific refractive increment of solids within the cell and for nucleated cells \(\alpha=0.002/n_{m}\) [6]. It is therefore worthy to note that the dry mass density can also be determined from the recorded hologram.

Figure 3 shows the dry mass density map of the 2 cell-stage embryos with an average dry mass of 4.2 g/dL and 6.7 g/dL for low- and high- lipid regions respectively again confirming quantitatively that the increase in RI is reflective of an increased in intracellular lipid content.

Fig. 3. Dry mass density maps of 2 cell-stage embryos cultured in low lipid (a) and high lipid (b) media. Scale bar: 30 µm.

3 Conclusion

In conclusion, DHM as a technique for assessing embryo quality has been presented and the results obtained from the reconstructed hologram showed good discrimination between embryos with different lipid contents. We have also shown that the retrieval of the dry mass density from the RI was possible and demonstrated a clear relation between the phase information obtained in DHM and the dry mass density.

The advantage in the use of DHM is that the 3D RI profiles can be obtained from a single-shot hologram as opposed to reconstruction from multiple 2D holograms recorded at various incident angles as used in other techniques such as optical diffraction tomography [7]. Its label-free nature also makes it ideal to combine with other imaging techniques for multimodal analysis. The low-power and non-invasive nature of this technique also highlights DHM as a potential imaging optical quality assessment technique for use in assisted reproduction clinics to augment conventional protocols.

References