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Determination of the Number of Cells in Preimplantation Embryos by Using Noninvasive Optical Quadrature Microscopy in Conjunction with Differential Interference Contrast Microscopy

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Abstract

The number of cells in a preimplantation embryo is directly correlated to the health and viability of the embryo. There are currently no methods to count the number of cells in late-stage preimplantation embryos noninvasively. We assessed the ability of optical quadrature microscopy (OQM) to count the number of cells in mouse preimplantation embryos noninvasively. First, to test for possible light toxicity, we exposed two-cell mouse embryos to OQM and differential interference contrast (DIC) microscopy and assessed their ability to develop to the blastocyst stage. We found no inhibition of development from either mode of microscopy for up to 2 h of light exposure. We also imaged eight-cell to morula stage mouse preimplantation embryos by OQM and developed two methods for counting the number of cells. The contour signature method (CSM) used OQM images alone and the phase subtraction method (PSM) used both OQM and DIC images. We compared both methods to standard cell counting techniques and found that the PSM was superior to all other noninvasive cell counting methods. Our work on mouse embryos should be applicable to human embryos. The ability to correctly count the number of cells in human preimplantation embryos could lead to the transfer of fewer embryos in in vitro fertilization (IVF) clinics and consequently a lower rate of high-risk multiple-infant births.

Keywords

cell counting; DIC; embryo viability; IVF; Keck 3DFM; light toxicity; OQM; quadrature; QTM

Introduction

There are two primary criteria that are used to evaluate the viability of preimplantation embryos: morphological features as judged by differential interference contrast (DIC) microscopy and the rate of development as measured by the number of cells in the embryos. Morphological features have been studied for many years, leading to the development of increasingly complex scoring systems that have been implemented by in vitro fertilization (IVF) clinics (for reviews, see Baczkowski et al., 2004; Rienzi et al., 2005). However, the success rates for pregnancy following IVF are still low. Despite over 2 million IVF babies born

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worldwide, the latest available statistics show that there is only a 34% pregnancy rate from IVF clinics in the United States (Centers for Disease Control, 2002), with a 30%–40% pregnancy rate common across the world. The inability to determine accurately which embryos are viable necessitates the transfer of more than one embryo per cycle to potential mothers. This, unfortunately, leads to a high rate of multiple-infant births and the many complications associated with multiple births. Based on statistics from the United States, approximately 29% of live births from IVF are multiple-infant births (Centers for Disease Control, 2002). The burden of multiple infants not only increases the health risks of pregnancy to the mothers, but also increases psychological, social, and economic stress for both parents. And the infants resulting from multiple-infant births suffer from problems such as increased respiratory distress syndrome, intracranial hemorrhage, cerebral palsy, blindness, and general increased morbidity and mortality. In fact, a twin is 7 times more likely and a triplet is 20 times more likely to die within the first month after birth (American Society for Reproductive Medicine, 2003).

The rate of development of an embryo has proven to be highly predictive of pregnancy success (for a review, see Baczkowski et al., 2004), but it has limitations. Rate of development is measured based on the number of cells in an embryo at a particular time after fertilization. Accurate methods for counting the number of cells in a preimplantation embryo include the Tarkowski method (Tarkowski, 1966), DNA synthesis measurement (Cozad et al., 1981), differential staining by immunosurgery (Handyside & Hunter, 1984), and Hoechst staining of the nuclei (Handyside & Hunter, 1984). These methods are invasive and result in the destruction of or damage to the embryo. However, by using DIC microscopy, which is the clinical standard for evaluating embryos, the number of cells becomes extremely difficult to count beyond the eight-cell stage (day 3 of development). Therefore, rate of development is measured solely on timing of the first cleavage or the number of cells on day 3 of development. As a result, most IVF clinics transfer day 3 embryos to patients and miss the opportunity to evaluate embryos during the two additional days in which the embryo can be cultured before transfer.

To count the number of cells noninvasively in late stage preimplantation embryos, a method capable of distinguishing the overlapping layers of cells is necessary. Optical quadrature microscopy (OQM), developed in our laboratory, measures both the amplitude and phase of light that passes through an object (Hogenboom et al., 1998; Glina et al., 1999; DiMarzio et al., 2001; Stott et al., 2001; Townsend et al., 2003). Therefore, OQM images include contributions from refractive index and absorption for all the cells in the light path. OQM complements DIC microscopy, which is particularly useful for distinguishing edge features in nearly transparent samples. Preliminary work has already demonstrated the ability of OQM to distinguish layers of cells in mouse preimplantation embryos (Townsend et al., 2003).

The purpose of this study was to determine whether OQM could be used to count the number of cells in mouse preimplantation embryos noninvasively. The first step in testing any new microscope for its potential to image live specimens noninvasively is to test for light toxicity. Two-cell mouse embryos were exposed to OQM and analyzed for subsequent development to the blastocyst stage. Development of an embryo to the blastocyst stage is a standard assay for testing toxicity (Nagy et al., 2003). As a control, embryos were also exposed to DIC microscopy, which is considered safe for live cell imaging. To determine the potential of OQM for counting cells, OQM was applied to mid-late-stage mouse preimplantation embryos. In this article, we describe two new methods for counting cells in mouse preimplantation embryos, the contour signature method (CSM) and the phase subtraction method (PSM).

Materials and Methods

A Note on Nomenclature

Earlier versions of what we are now calling the OQM were named the quadrature tomographic microscope (QTM)(DiMarzio et al., 2001; Stott et al., 2001; Townsend et al., 2003) because of the potential for doing tomographic imaging. We have recently determined that tomography is unnecessary for our cell counting purposes, although the potential for doing tomography remains. Therefore, we have decided to use OQM rather than QTM to describe the microscope.

Testing of Light Toxicity by DIC and OQM

Power and Wavelength Measurements—An Ocean Optics S2000 spectrophotometer (Dunedin, FL) was used to measure the spectrum of light illuminating the embryos from the light sources of the OQM and the DIC microscopes. A black cover was placed over the microscope and the meter was placed on the microscope stage in the same plane in which the specimen would be located.

A power meter was used to measure the power of light at the level of the specimen. Peak irradiance was calculated by dividing the mean power level by the diameter of the beam. A different power meter was required for each modality due to the wavelength limitations of the meters. A Newport 1830C optical power meter (Santa Ana, CA) was used with OQM, and a Spectra-Physics 407A power meter (Mountain View, CA) was used with DIC microscopy.

Mice and Embryo Collection—C57BL/6J and CBA/CaJ strains of mice were purchased from the Jackson Laboratory (Bar Harbor, ME) and bred in our laboratory. The mice were handled according to the Institutional Animal Care and Use Committee (IACUC) regulations in an Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) approved facility. C57BL/6 female mice, 2–6 months old, were superovulated according to standard procedures (Nagy et al., 2003). A first injection of 5 IU of eCG (Sigma, St. Louis, MO) was administered, followed 48 h later by 10 IU of hCG (Sigma). Individual C57BL/6 female mice were mated overnight with a single CBA/Ca male and evaluated for the presence of a vaginal plug the next morning, indicating that mating had occurred. Two-cell embryos were collected from plug-positive females the day after plug detection. The embryos from all mice were pooled, washed, and placed in a dish containing M2 medium (Specialty Media, Phillipsburg, NJ).

Exposure to Light Microscopy—Mat-Tek coverslip-bottomed culture dishes (Mat-Tek, Ashland, MA) were prepared containing two 10- μ l drops of M2 medium under medium-equilibrated mineral oil (Fisher Scientific, Pittsburgh, PA). Ten two-cell embryos were placed in each drop. One drop in each dish was marked as a control (no light exposure) and one drop was marked as the test.

For DIC microscopy, a Mat-Tek dish containing two-cell embryos was placed on the stage of an inverted microscope. The setup, which represented typical DIC imaging conditions for preimplantation embryos, remained unchanged between experiments. The two-cell embryos were located in the test drop, by using a 20 $\times$ DIC objective, and then centered in the field of view. The DIC polarizer and analyzer were pushed into place, and the timed exposure was started. At the end of the designated exposure time, the DIC polarizer and analyzer were removed from the light path, and the culture dish was removed from the microscope stage. The next culture dish was placed on the microscope, and the exposure procedure was repeated. Several dishes of varying exposure lengths were tested during each experiment.

For experiments with OQM, a Mat-Tek dish containing two-cell embryos was placed on the microscope stage. Using the tungsten light source, the two-cell embryos were located in the test drop and centered in the field of view. This procedure took less than 30 s. The tungsten light source was turned off, the OQM 633 nm laser was switched on, and the timed exposure was started. At the end of the designated exposure time, the laser was switched off and the culture dish was removed from the microscope stage. The next culture dish was placed on the microscope and the exposure procedure was repeated. Several dishes of varying exposure lengths were tested during each experiment.

Culture to Blastocyst Stage—Embryos from the test and control drops were placed in Falcon center-well organ culture dishes (Becton Dickinson, Franklin Lakes, NJ) containing two 10- μ l drops of Human Tubal Fluid (HTF) medium (Irvine Scientific, Santa Ana, CA) under medium-equilibrated mineral oil. Thus, the control and test embryos from each time exposure were allowed to develop in the same dish. After addition of the embryos, the plates were returned to the incubator and cultured for 72 h. The stage of development was recorded for each embryo at the end of the culture period.

Statistical Analyses—All statistical analyses were performed using SPSS standard version 11.0 software for the PC (Chicago, IL).

Counting Cells in Embryos by DIC and OQM

Mice and Embryo Collection—C57BL/6 female mice were superovulated, as described previously, and mated with single CBA/Ca male mice. Plug positive female mice were sacrificed on days 3 and 4 post-hCG injection and eight-cell through morula stage embryos were collected in M2 medium. The embryos from each mouse were stained and imaged before the following mouse was sacrificed in order to minimize the amount of time the embryos spent outside of the mouse. Embryos spent less than 60 min outside of the mouse or an incubator.

Staining and Imaging of Embryos—Embryos were stained 30 min in 1 μ g/ml Hoechst 33342 (Serologicals Corporation, Norcross, GA). Morphologically normal appearing embryos (no visible fragmentation, intact/healthy appearing zona pellucida and cells) were placed in M2 microdrops, under equilibrated oil, in a Mat-Tek coverslip-bottomed imaging dish. Dishes were placed on a Nikon TE200 OQM equipped microscope that was fitted with a 20 $\times$ 0.45 NA objective lens. DIC, UV illuminated epifluorescent, and OQM center-focus images and 10 μ m z-stacks of images were collected for each embryo. Images were collected using MATLAB software (The Mathworks, Inc., Natick, MA), and they were converted to TIF files for visualization and counting cells manually.

Counting Cells Manually—All TIF images were opened in Metamorph software (Molecular Devices Corp., Downingtown, PA). The “Manually Count Objects” function was used to hand-count and log the number of cells visible in DIC and OQM images. The number of cells for each embryo was counted using the epifluorescent images of Hoechst-stained nuclei. Polar bodies were identified by size and location, and they were counted separately from the embryonic cells.

Counting Cells by Using the Contour Signature Method—In MATLAB, OQM images were first normalized to an intensity scale of 0–1.0. They were then analyzed to determine the number of cell layers based on the optical path length deviations. Thresholds were applied to define the boundary for each layer. Then the boundaries were edge-enhanced using a Sobel edge filter. Morphological operations, in particular an open operation, erosion followed by dilation, were used to fill in holes in the images. A distance transform, using Euclidean geometry, was then applied to fill in the boundary and define the centroid. The

contour signature curve was then created by plotting the distance between the centroid and each point along the boundary. The curve was denoised by using a Daubechies Wavelet (order 3), and each maximum was counted as a cell.

Counting Cells by Using the Phase Subtraction Method—In MATLAB, OQM and DIC images were first registered with an affine transformation. An outer cell with a smooth optical path length deviation (OPD) and a small amount of cell overlap was chosen to start the process. A profile line was used to plot the OPD through the cell and a parabola was fitted to the curve. This defined the OPD for a single cell. An ellipse was created using the center, minimum radius, and maximum radius of a cell boundary from the DIC image. The ellipse, combined with the OPD, was used to create an ellipsoidal model of that cell, which was then subtracted from the OQM image. Elliptical boundaries were created in turn for all cells clearly visible in the DIC image, and these cells were then modeled in the same fashion and subtracted from the OQM image. The subtracted OQM image was then analyzed for “hidden” cells not seen in the DIC image. These “hidden” cells were modeled and subtracted from the OQM image until no embryonic cells remained.

Results

Our first goal was to determine the potential toxicity of OQM as compared to DIC microscopy. Wavelength and power measurements were made for the OQM and DIC microscopes with the results shown in Table 1. The spectrophotometer confirmed that the 633-nm laser used in OQM produces a sharp peak of 633 nm light at the specimen level. In contrast, the spectrophotometer measured a broad range of wavelengths at the specimen level in the DIC microscope. In addition to producing a wider range of wavelengths, the DIC microscope produced a higher peak irradiance than the OQM.

In two separate but parallel experiments, two-cell embryos were exposed to OQM (total $N = 861$) or DIC (total $N = 1091$) microscopy imaging conditions for 0–120 min (Tables 2 and 3). The number of nonimaged control embryos and the number of test embryos to reach the blastocyst stage were recorded and converted to percentages. The percentage of cells that developed to the blastocyst stage after light exposure was compared to the controls for each time point in order to account for possible plate-to-plate and day-to-day variation. Percentages above 100% of control demonstrate more frequent development to the blastocyst stage by the test group than the control group, and numbers below 100% of control demonstrate a deficiency in development to the blastocyst stage as compared to the control group. The numbers are expressed as mean $\pm$ standard error of the mean (*SEM*).

The results of embryo exposure to DIC microscopy are presented in Table 2. No significant differences between control (no light exposure) and test (light exposed) embryos were found at any time point by Student's *t* test or by ANOVA across all time points. Table 3 presents the equivalent results for embryos exposed to OQM. Embryos exposed to OQM were not significantly different from control embryos at any time point or across all time points. Student's *t* test at each time point also demonstrated no significant differences in development to the blastocyst stage between embryos exposed to the DIC light source and embryos exposed to the OQM light source. An ANOVA performed to analyze the effects of the type of light (DIC or OQM) across all exposure times for the control and test groups also showed no significant differences between any of the groups.

Our second goal was to determine whether analysis of OQM images could be used to determine the number of cells in mouse preimplantation embryos. Embryos were stained with Hoechst to identify the nuclei, and then imaged by DIC, epifluorescence, and OQM. Hoechst staining did not have an effect on OQM images, as the images collected from unstained and stained

embryos appeared the same. The number of cells in each embryo was counted blindly among the different modalities, meaning that the information from one modality was unavailable when counting cells in another modality. Figure 1 shows examples of an embryo with a low number of cells ($N = 12$) and an embryo with a high number of cells ($N = 21$) as determined by epifluorescent imaging of the stained nuclei (Fig. 1A,D). Because Hoechst staining is an accepted standard for counting the number of cells in a sample, we used the epifluorescence cell count as the true number of cells. We did not see any evidence that the number of nuclei did not match the actual number of cells. Cells were counted manually by DIC (Fig. 1B,E) and OQM (Fig. 1C,F). The first embryo was counted correctly as 12 cells by all modalities, but the second embryo cell count was underestimated by both DIC and OQM manual cell counting.

Figure 2 shows the results of using the CSM to count the number of cells in OQM images of embryos. Figure 2A–E shows the results for the 12-cell embryo, and Figure 2F–K shows the results for the 21-cell embryo that were shown in Figure 1. OQM images (Fig. 2A,F) show the initial thresholding to eliminate the background and isolate the embryo (Fig. 2B,G). Edge enhancement and the open operation produced a binary image of the isolated embryo with a sharply defined boundary (Fig. 2C,H). Generation of the contour signature curve for the first layer (Fig. 2D,I) identified the majority of the cells in the embryos. After applying a second threshold and repeating the CSM, a second contour signature curve was generated to identify overlapped cells (Fig. 2E,J). In embryos with a high cell number, a third application of the CSM with another threshold resulted in a final contour signature curve (Fig. 2K). By counting the maxima on the curves, the total cell number was determined. Polar bodies were excluded from the cell counts based on their size. The CSM correctly identified the 12 cells in the first embryo, but it incorrectly counted 20 cells in the second embryo.

The PSM was used to count the number of cells by using both OQM and DIC images. Figure 3A–E shows the results for the 12-cell embryo, and Figure 3F–J shows the results for the 21-cell embryo that were shown in Figure 1. Using the OQM image to start, a line was drawn through one cell of an embryo (Fig. 3A,F) and a plot of the optical path length deviation (OPD) along the line was created (Fig. 3B,G). We could easily identify and label the transitions between structures on the plot. An ellipse was fitted to the chosen cell (Fig. 3C,H) on the DIC image. By using the information generated from the OPD plot and the ellipse, the cell was subtracted from the OQM image (Fig. 3D,I). The boundaries of overlapped cells were revealed, and the process was repeated until all cells were subtracted (Fig. 3E,J). Polar bodies were again excluded from the cell counts based on their size. The PSM correctly counted 12 cells for the first embryo and 21 cells for the second embryo. Movies of the phase subtraction method may be viewed at: <http://www.keck3dfm.neu.edu/samplevideo>.

We compared the cell counting methods in 15 embryos by calculating the average error for each method compared to the “ground truth” cell number determined by Hoechst staining of nuclei. The results are shown in Table 4. The error represents the average number of cells ($\pm SEM$) each method over- or underestimated the number of cells in the embryos. The PSM demonstrated almost a 10-fold lower error than other cell counting methods, and it was able to produce an accurate count of the number of cells in embryos with as many as 26 cells.

Discussion

OQM provides a new method to noninvasively image unstained, transparent biological specimens such as preimplantation embryos. In this article, our first experiments were designed to measure the effect of light exposure from OQM on development of two-cell mouse embryos cultured to the blastocyst stage. The wavelength, power, and exposure time of a biological specimen to light determines the level of damage to that sample. As a result, previous studies on oocyte viability and embryo development after exposure to light have resulted in different

conclusions, with some studies reporting no effect (Kruger & Stander, 1985; Sauer et al., 1991; Barlow et al., 1992) and other studies reporting a variety of impediments, including inhibition of cleavage and cell death, to oocyte and embryo development (Daniel, 1964; Hirao & Yanagimachi, 1978; Fischer et al., 1988; Hegele-Hartung et al., 1988, 1991; Schumacher & Fischer, 1988; Nakayama et al., 1994; Squirrell et al., 1999). To properly evaluate the potential toxic effects of OQM, we first measured the wavelength and power at the specimen level. As shown in Table 1, OQM exposes embryos to a fivefold lower intensity of light than DIC microscopy, which is considered safe for live cell imaging. Embryos exposed to DIC light microscopy demonstrated no significant decrease in the percentage that developed to the blastocyst stage (Table 2). Likewise, OQM showed no indication of impairing the capacity of early mouse embryos to develop to the blastocyst stage (Table 3). OQM uses a red-light helium-neon laser (633 nm wavelength) as the illumination source, thereby reducing damaging violet and blue wavelengths of light. Wavelengths of light in the red region have been reported to be relatively nondamaging (Daniel, 1964; Hirao & Yanagimachi, 1978). In fact, red light has been shown to increase cytochrome c oxidase activity and transcription of genes associated with energy production (Eells et al., 2003). This may account for the consistent, although not statistically significant, increase in the percentage of blastocysts developing from OQM-exposed embryos compared to non-exposed embryos and DIC-exposed embryos. The length of exposure times used was sufficient to evaluate potential toxicity because observation of embryos with OQM only requires exposures on the order of minutes.

The real power of OQM is that it can view information on the inside of a specimen. Specifically, the ability to distinguish layers of cells provides the first opportunity to count the number of cells in late-stage preimplantation embryos noninvasively. For comparison to OQM cell counting methods, we counted the actual number of cells by Hoechst staining and counted cells by DIC as a representation of the best current technique for noninvasive cell counting. Counting by hand was subjective and time-consuming, as well as inaccurate, particularly for high cell number embryos. We developed two novel methods to more objectively analyze the number of cells in preimplantation embryos. The first, CSM, had been used in a preliminary version with promising results, but the errors were quite high (Warger et al., 2005). Advancements in thresholding were applied in the present study, thus reducing the CSM error. However, CSM remained inaccurate and time-consuming. By utilizing both DIC and OQM images, we developed the second method, PSM. PSM is both accurate and precise, as demonstrated by the low *SEM* (Table 4). Cells were readily identified by using DIC to locate the cell boundaries and by using OQM to model the phase contribution of the cell. However, it should be noted that the 15 embryos evaluated in this study had clear cell boundaries. Samples with image defects or mostly occluded cell boundaries are still too difficult to analyze with the current methods. Further work to automate and speed up the PSM should allow us to process samples with defects or occluded boundaries and to perform a more comprehensive analysis that could include identifying and counting polar bodies and fragments. Improving our algorithms should allow us to process samples in minutes instead of hours, making this technique more likely to be applicable to clinical use.

Our research on cell counting in mouse preimplantation embryos has the potential for application to the IVF clinic. Despite criticisms that longer culture may induce defects due to culture conditions, there is evidence that allowing embryos to develop past the eight-cell stage may lead to better embryo evaluation and natural elimination of the nonviable embryos (Rijnders & Jansen, 1998; Tao et al., 2002). Advancements in cell counting methods and their implementation would give clinicians the ability to better recognize high-cell-number embryos just prior to their development into blastocysts or even to extend this technique to blastocysts themselves. Identification of a single healthy morula or blastocyst by using morphological characteristics and the number of cells per embryo could give clinicians the best chance for

increasing single Embryo Transfer (sET) success rates. There is a worldwide effort for sET in order to minimize the frequency of multiple-infant births (Gurgan & Demiroglu, 2004).

Finally, a recent advancement in OQM has been the addition of OQM to the Keck three-dimensional fusion microscope (3DFM), an advanced multimodal microscope built and housed at Northeastern University (Townsend et al., 2005). OQM is a relatively inexpensive add-on that could be adapted to standard microscopes and used with any desired magnification. Addition of OQM to the Keck 3DFM gives us the opportunity to combine the potential of OQM with other advanced imaging modalities such as laser scanning confocal microscopy and multiphoton microscopy to develop and evaluate further noninvasive imaging techniques.

Conclusions

We have demonstrated that by using OQM in conjunction with DIC microscopy we have greatly increased our ability to accurately count the number of cells in mouse preimplantation embryos in a noninvasive manner. Two cell counting methods have been developed, one using OQM images alone (contour signature method) and the other using both OQM and DIC images (phase subtraction method). The cell counting methods we developed using mouse embryos could be applied to human embryos in the IVF clinic. This could further the worldwide goal of the transfer of one healthy embryo to the recipient mother, thereby increasing the pregnancy rate after IVF and simultaneously decreasing the morbidity and mortality associated with multiple-infant births.

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Figure 1.

Counting the number of cells in embryos manually. Images **A–C** are from a 12-cell embryo and images **D–F** are from a 21-cell embryo. The actual number of cells in each embryo is represented by the count from the epifluorescence images of the Hoechst stained nuclei (**A,D**). The number of cells counted manually in the DIC images is shown at the bottom right-hand corner of **B** and **E**, and the number of cells counted manually from the OQM images is shown at the bottom right-hand corner of **C** and **F**. The color bar on the OQM images represents the optical path length deviation (OPD).

Figure 2.

Counting the number of cells in embryos by using the contour signature method (CSM). Images A–E are from the 12-cell embryo and images F–K are from the 21-cell embryo shown in Figure 1. OQM images (A,F) were first thresholded (B,G), edge-enhanced (C,H), and then a distance transform operation was performed. Contour signature curves (D,E,I–K) were generated for each layer of cells. The x -axis represents the pixels along the perimeter and the y -axis represents the distance from the center of the embryo. Each maxima (marked with a red +) indicates a cell. The resulting number of cells, by using the CSM, is shown at the bottom of each column.

Figure 3.

Counting the number of cells in embryos by using the phase subtraction method (PSM). Images **A–E** are from the 12-cell embryo and images **F–J** are from the 21-cell embryo shown in Figure 1. Lines were drawn through a single cell on the OQM images (**A,F**), which were used to generate plots of distance along the line versus OPD (**B,G**). The plots were used in conjunction with the DIC images to generate elliptical boundaries for the cells (**C,H**). The ellipses were used to sequentially subtract cells from the OQM images (**D,I**). At the end of the process, the polar body was the only remaining cell in the OQM images (**E,J**). The resulting number of cells, by using the PSM, is shown at the bottom of each column.

Table 1

Spectra and Power Measurements for DIC and OQM Light Microscopy

Imaging Method	Light source	Wavelength (nm) ^a	Peak irradiance (W/cm ²) ^b
DIC	50-W tungsten halogen	500–760	3×10^{-3}
OQM	15-mW 633-nm laser	633	6.6×10^{-4}

^aWavelength was measured using an Ocean Optics S2000 spectrophotometer.

^bPeak irradiance was measured using a Newport 1830C optical power meter for OQM and a Spectra-Physics 407A power meter for DIC.

Table 2

Embryo Development after DIC Light Exposure^a

Exposure time (min)	No. of replicates	No. of control embryos ^b	Blastocyst formation (%) ^c	No. of Test embryos	Blastocyst formation (%) ^c	Percentage of control ^d
0	2	24	91 ± 13	24	87 ± 13	96 ± 19
0.5	4	78	71 ± 8	78	67 ± 8	95 ± 11
2	4	70	71 ± 9	70	67 ± 9	99 ± 11
5	4	80	74 ± 8	77	63 ± 8	90 ± 12
10	4	90	68 ± 8	90	62 ± 8	96 ± 11
20	6	116	73 ± 6	116	76 ± 7	90 ± 11
60	4	43	79 ± 10	45	76 ± 10	94 ± 15
120	4	45	96 ± 10	45	98 ± 10	100 ± 19

^aEmbryos were collected at the two-cell stage, subjected to various exposure times using DIC microscopy, and cultured as described in the text.

^bControl embryos were not exposed to light.

^cMean ± SEM.

^dNumber of test embryos that developed to blastocyst is expressed as a percentage of the number of control embryos that developed to blastocyst ± SEM.

Table 3

Embryo Development after OQM Light Exposure^a

Exposure time (min)	No. of replicates	No. of control embryos ^b	Blastocyst formation (%) ^c	No. of test embryos	Blastocyst formation (%) ^c	Percentage of control ^d
0	1	34	89 ± 6	35	93 ± 6	106 ± 12
0.5	3	56	77 ± 5	60	80 ± 5	113 ± 10
2	3	67	82 ± 4	65	86 ± 4	104 ± 8
5	2	50	80 ± 5	50	82 ± 5	104 ± 11
10	2	50	78 ± 5	50	86 ± 5	111 ± 11
20	5	77	87 ± 4	76	90 ± 4	103 ± 9
60	2	47	89 ± 5	50	94 ± 5	107 ± 11
120	2	50	88 ± 5	50	94 ± 5	118 ± 14

^aEmbryos were collected at the two-cell stage, subjected to various exposure times using OQM, and cultured as described in the text.

^bControl embryos were not exposed to light.

^cMean ± SEM.

^dNumber of test embryos that developed to blastocyst is expressed as a percentage of the number of control embryos that developed to blastocyst ± SEM.

Table 4

Comparison of Cell Counting Methods

	No. embryos analyzed	Range in cell no.	Error for all embryos ^a (N = 15)	Error ≤12 cells ^a (N = 5)	Error > 12 cells ^a (N = 10)
DIC ^b	15	8–26	–1.0 ± 1.5	0.6 ± 1.4	–1.8 ± 1.3
OQM ^b	15	8–26	–0.9 ± 1.8	2.6 ± 1.6	–2.6 ± 1.5
CSM ^c	15	8–26	–1.9 ± 1.9	–0.4 ± 1.4	–2.6 ± 2.1
PSM ^d	15	8–26	–0.1 ± 0.6	0	–0.2 ± 0.6

^aThe error is mean ± SEM of the individual errors for embryos as calculated by the number of cells counted minus the actual number of cells (determined by Hoechst staining). The error is presented as total error, the error for embryos with ≤12 cells, and the error for embryos with > 12 cells.

^bImages were opened in Metamorph and the number of cells was counted manually.

^cOQM data files were opened, and the contour signature method was applied as described in the text.

^dOQM data files were opened, and the phase subtraction method, using an elliptical fit model on DIC images, was applied as described in the text.