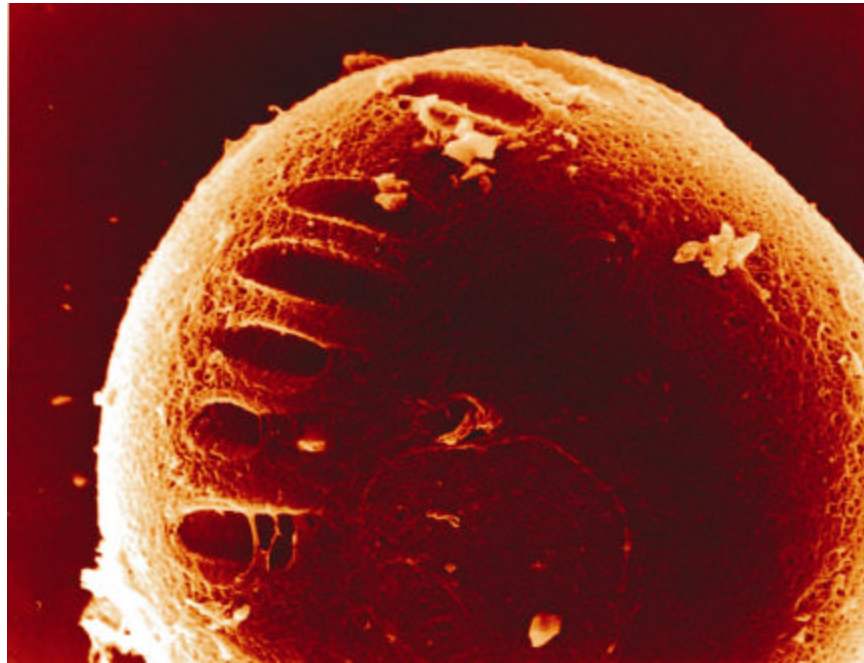


# ZILOS

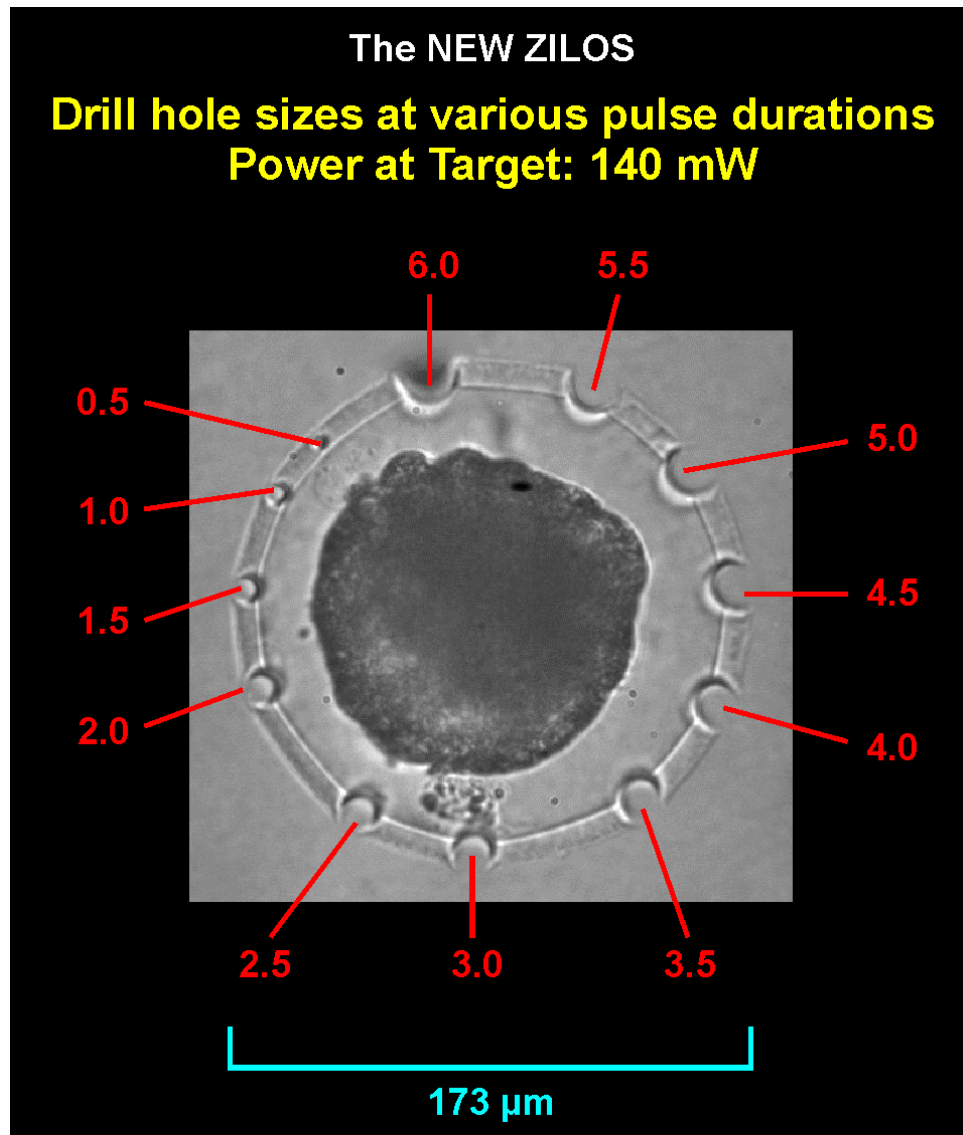
(Zona Infrared Laser Optical System)

## Focus on Heat Diffusion and Embryo Safety During Laser-Assisted Zona Dissection



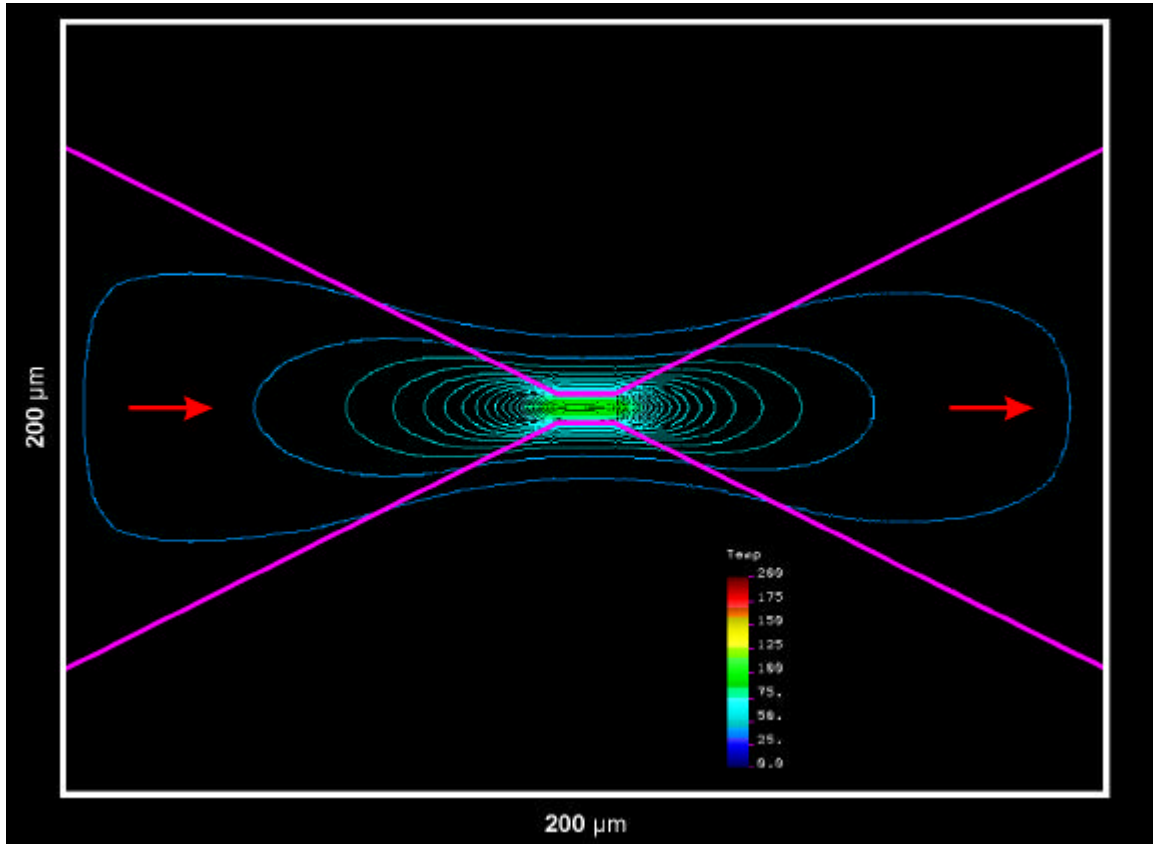
100 Cummings Center, Suite 102C, Beverly, MA 01915  
800-323-0503, 978-921-2050, Fax: 978-921-0250  
sales@hamiltonthorne.com, www.hamiltonthorne.com/research  
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The size of the hole in the zona is directly related to the duration of the laser pulse, as shown in Figure 1. The longer the pulse duration, the larger the drill hole size. However, longer pulse durations also mean increased heat generation. Thus, it is much safer to make several short pulses to create a good hole. The most important thing is the safety of the embryo.



*Figure 1. Drill Hole Sizes at Various Pulse Durations with Power at Target of 140 mW (Bovine Oocyte).*

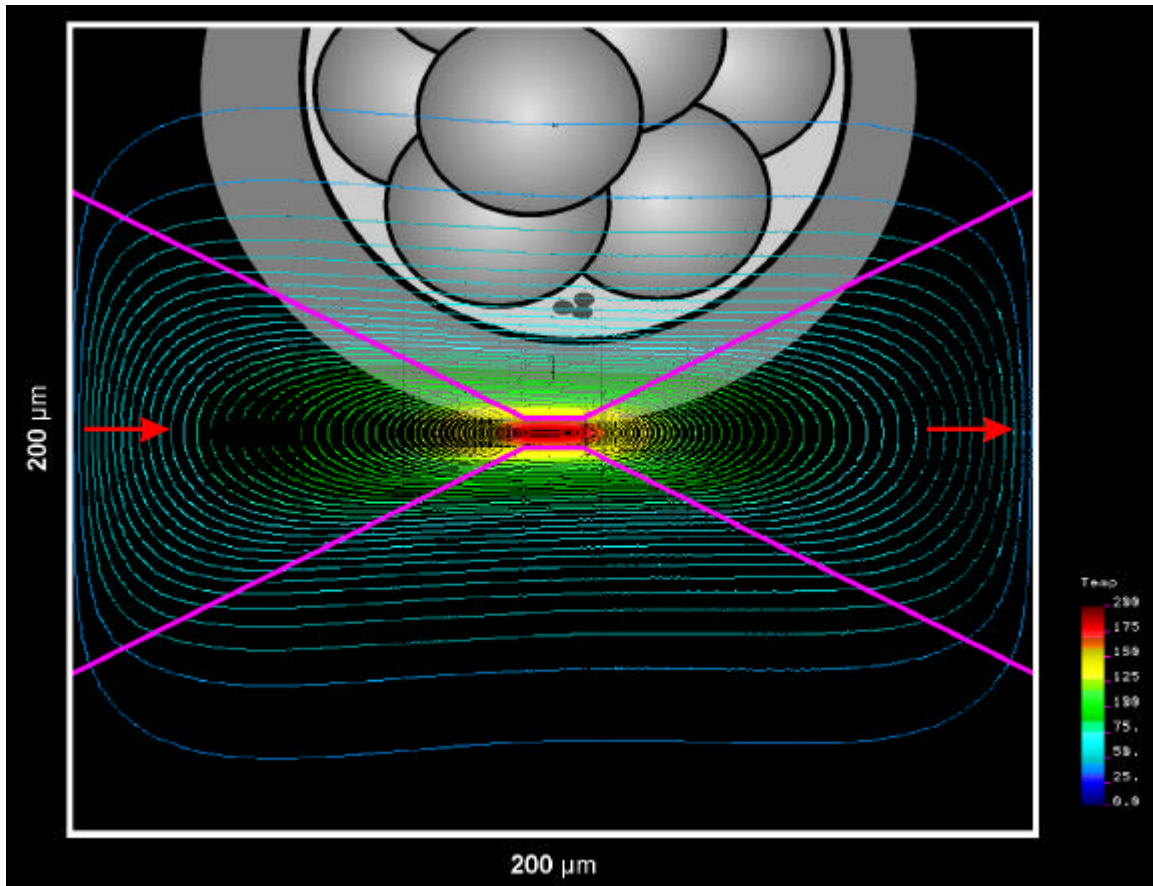
The common assumption was that the heat from the laser diffuses spherically. This assumption is incorrect. Actually, the heat diffuses in a cylindrical shape, resulting in higher temperatures in the beam center (Figure 2).



*Figure 2. Heat Diffusion at  
Power = 100 mW  
Pulse Length = 0.1 ms*

*The contours are lines of constant temperature at 0.1ms.*

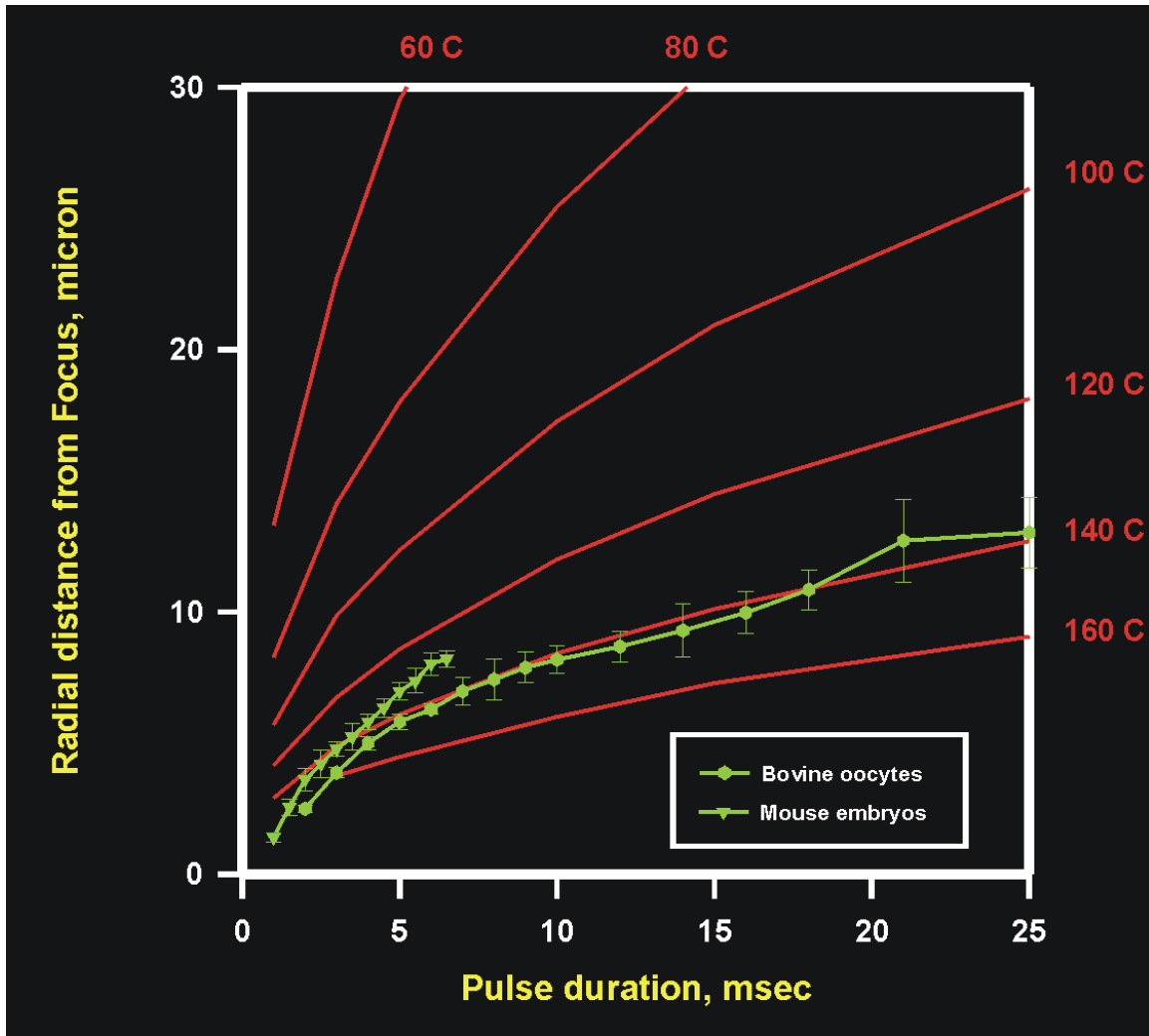
As shown below in Figure 3, the diffusion of heat, even at pulse duration 3 ms with a power of 100 mW, clearly extends into the embryo and may damage nearby cells. Most commercial lasers use similar or longer pulses.



*Figure 3. Heat Diffusion at  
Power = 100 mW  
Pulse Duration = 3 ms*

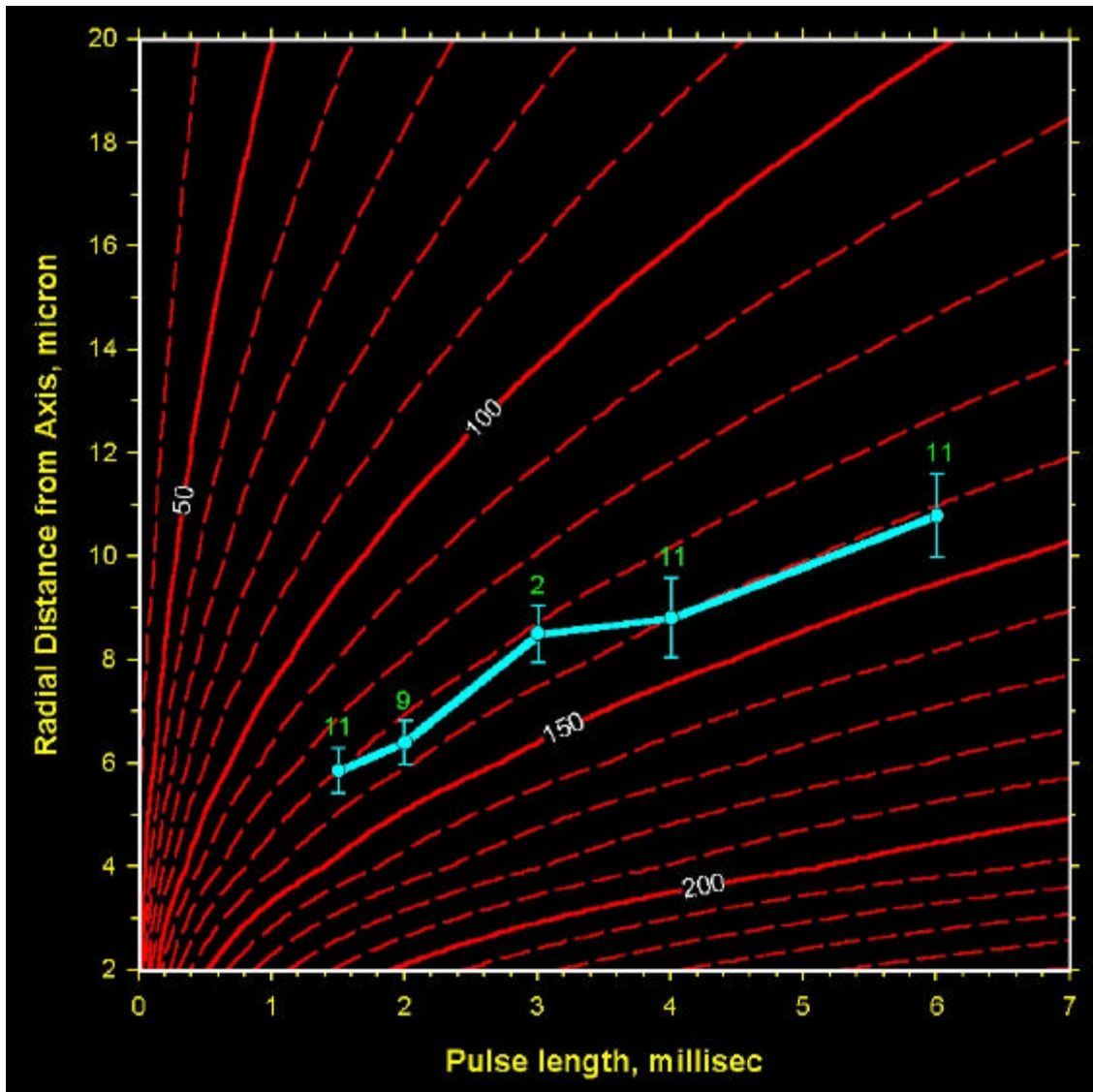
*The maximum temperature reached, during a 3 ms pulse with power at target of 100 mW, is shown by the contours.  
The contours are separated by approximately 2.5°C.*

The common assumption was that the zona “melts” at 100°C. This assumption is incorrect. The zona actually “melts” (undergoes denaturation) at 140°C. The graph in Figure 4 shows both mouse and bovine embryos have a similar denaturation pattern along the 140°C line.



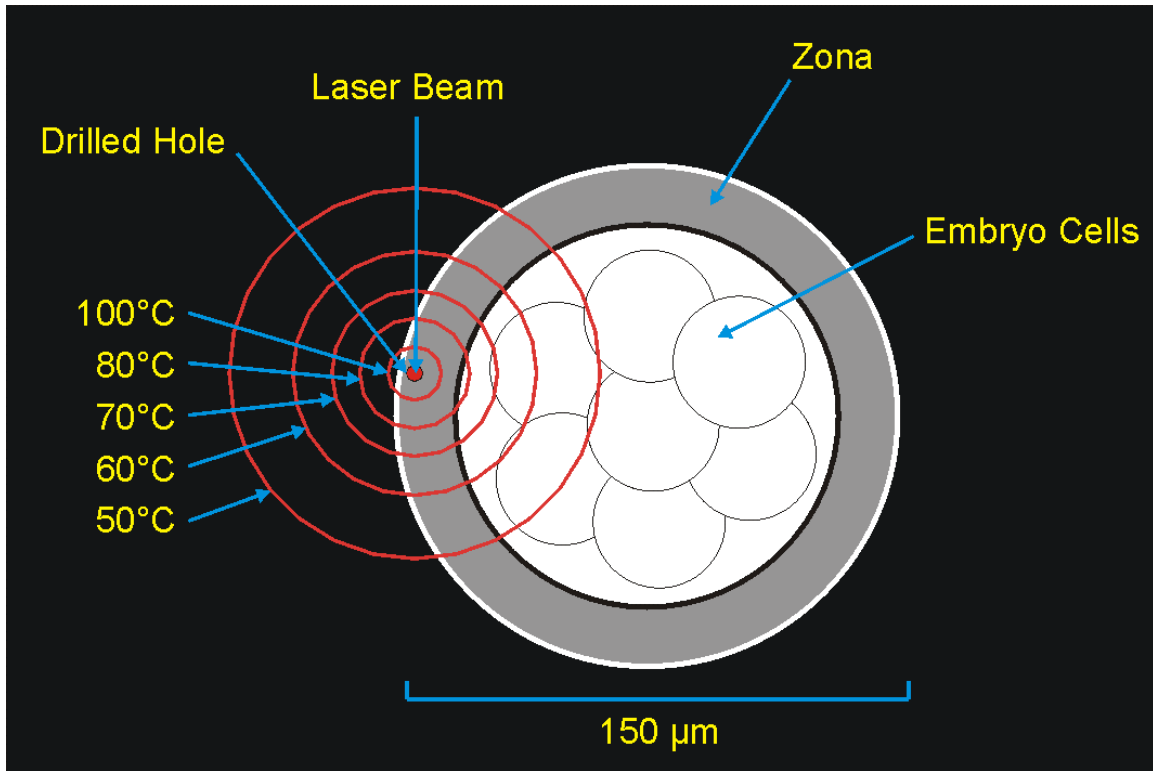
*Figure 4. Bovine and Mouse Zona Penetration at 100 mW Power (2 Bovine Oocytes, 2 Mouse Embryos)*

As demonstrated by Dr. Susan Lanzendorf at the JONES INSTITUTE, the melt line for human zona pellucida is also at 140°C (Figure 5).



*Figure 5. Peak Temperature and Zona Drilled Radius  
Laser Power @ Target = 140 mW  
Human zona pellucida hole size shown in blue.*

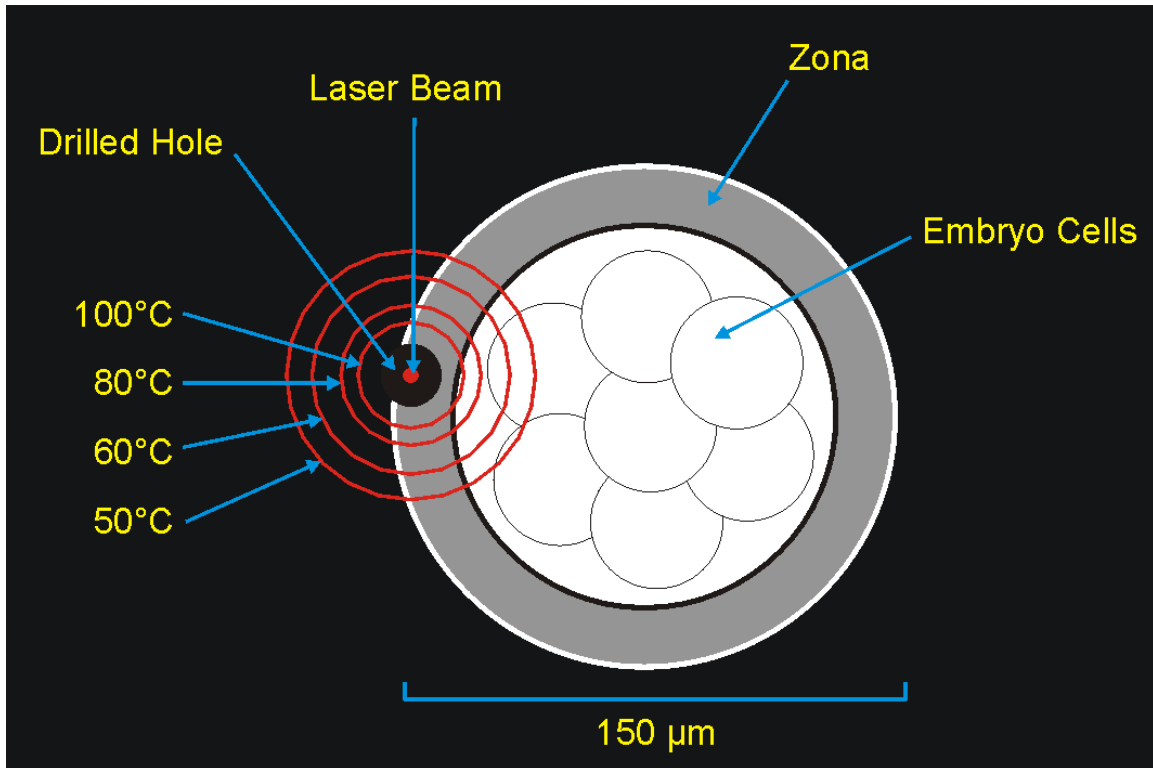
Figure 6 is an important diagram showing the maximum temperature reached at different diameters for pulse duration 25 ms and power at target 45 mW. This regime is potentially dangerous to the surrounding cells.



*Figure 6. CRI & Other Lasers  
Power = 45 mW  
Pulse Duration = 25 ms*

The diagram in Figure 7 shows that great care must be taken if you use a pulse length as long as 3 ms, as there is danger of damage to nearby cells.

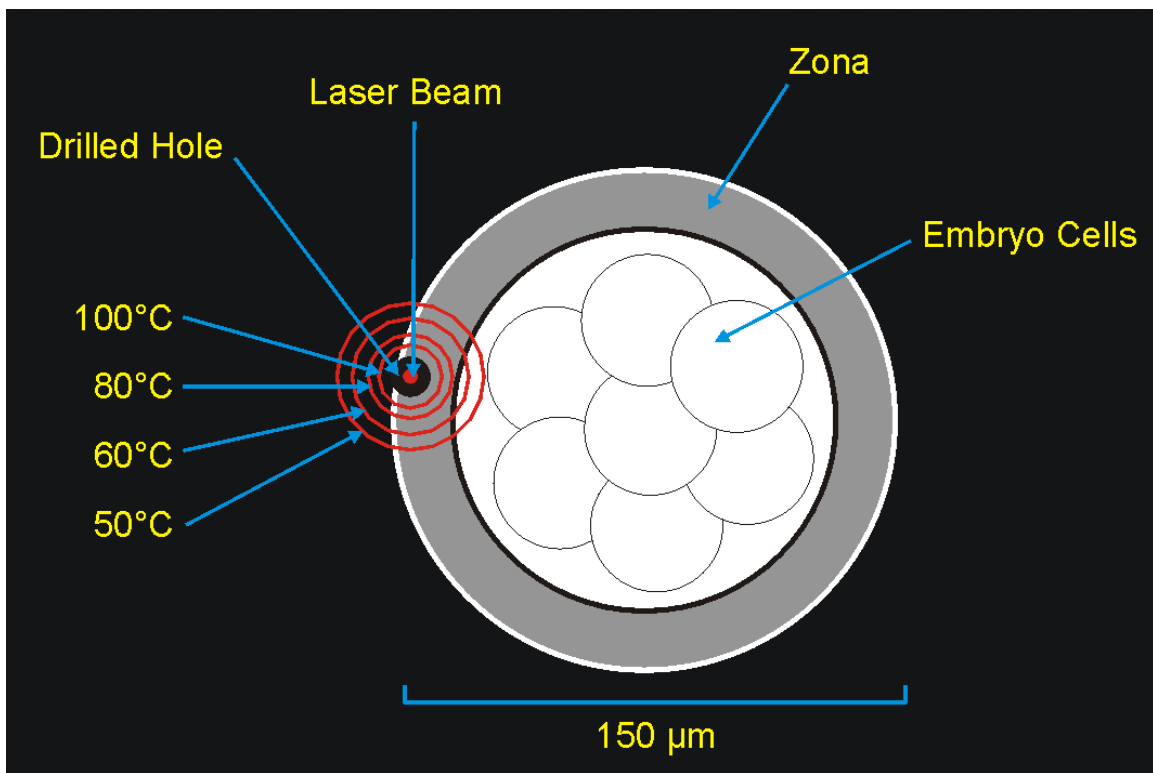
It is important to realize that most laser manufacturers tell you the power of the beam at the laser source. Absorption in the medium causes the power at the source to be very different from the laser power at the zona. The ZILOS has a power of 200 mW at the laser source, and 140 mW at the zona surface.



*Figure 7. Laser and Embryo Temperature  
Power at Target = 140 mW  
Pulse Duration = 3 ms*

The diagram in Figure 8 shows the safe regime of the ZILOS with 140 mW power at target and 1 ms pulse duration. It is clear that the hole is a good size, and that selection of a drilling location at an area where there are no cells touching the zona will be safest for the blastomeres.

It is easy to see that several 1 ms pulses can create a large hole in the zona while avoiding damage to the blastomeres. With a living embryo, it is worth taking a few extra seconds to increase the size of the hole if such proper treatment improves your research or fertility success.



*Figure 8. ZILOS Laser and Embryo Temperature  
Power at Target = 140 mW  
Pulse Duration = 1 ms*

The graph in Figure 9 depicts the international definition of a Class I laser at wavelength  $\lambda = 1480$  nm. You can see that all the ZILOS' power and pulse combinations are within the Class I laser boundary. A Class I laser frees your laboratory from regulatory and other restrictions.

The ZILOS is the only Class I laser currently on the market for zona dissection.

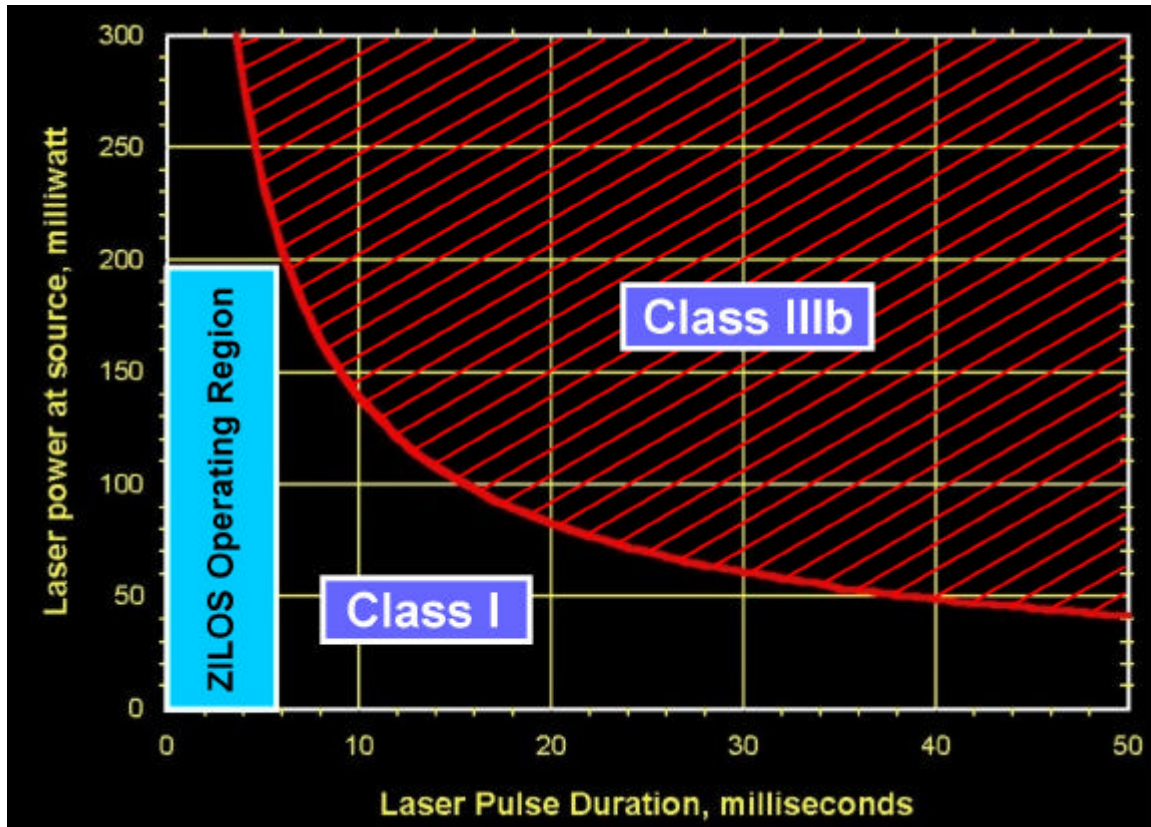
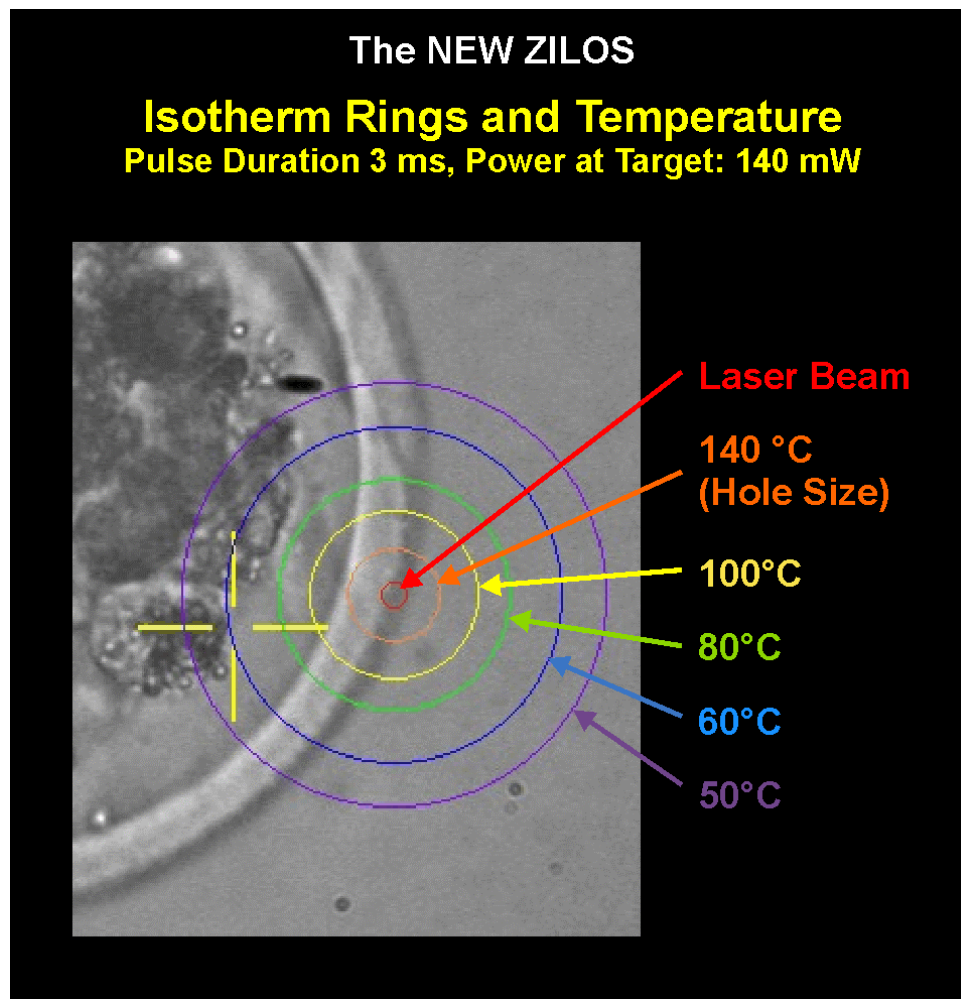


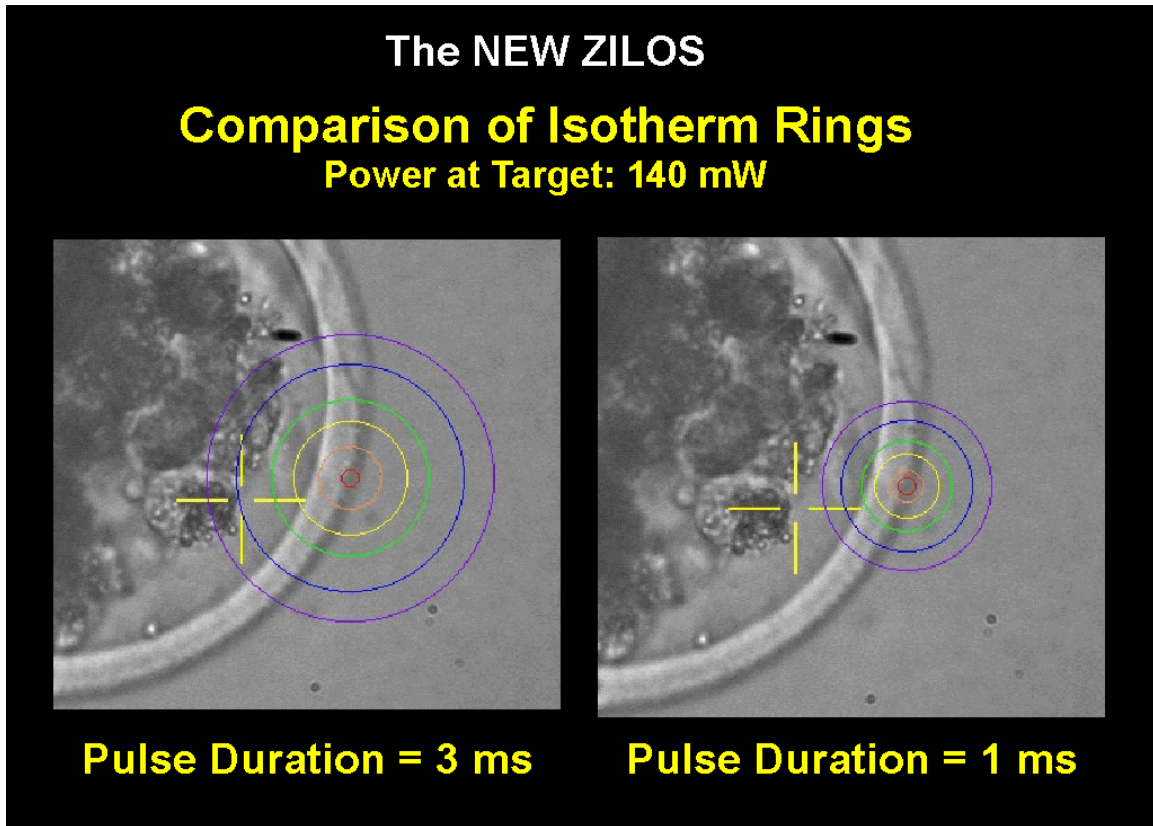
Figure 9. Class I – Class IIIb Boundary (FDA 1999)

The unique *Isotherm Rings* feature on the ZILOS helps you prevent potential harmful effects on blastomeres adjacent to the zona due to the heat from the laser drilling. The *Isotherm Rings* appear on the screen as a series of six concentric circles of varying colors, and indicate the maximum temperature reached at the ring diameter at various laser pulse durations. The orange ring (second from center) also indicates the drill hole size at the selected pulse duration.



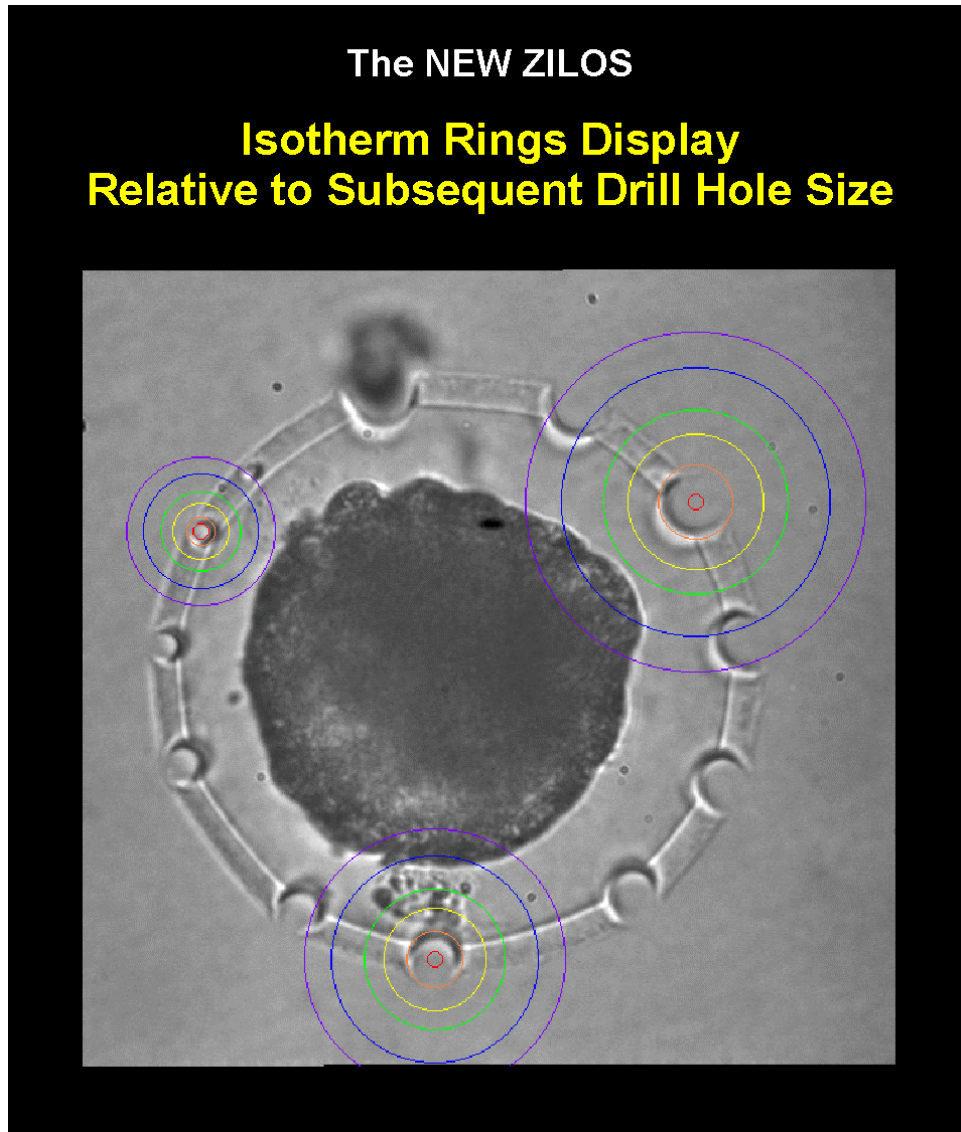
*Figure 10. ZILOS Isotherm Rings  
(3 ms pulse used for reference purposes only: laser was not fired).  
The same ring temperature color scheme is used for all diagrams*

With this interactive *Isotherm Rings* feature, users can now “see” the maximum temperature and drill hole size and eliminate the temperature “guesswork.”



*Figure 11. Comparison of Isotherm Rings at 3 ms and 1ms Pulse Duration  
(Power at Target = 140 mW)*

Figure 12 shows the Isotherm Rings relative to the drill hole size (same bovine oocyte as previously shown in Figure 1). Notice how for each hole the orange ring ( $140^{\circ}\text{C}$ ) matches the resultant drill hole size. Also, compare the temperatures reached near the beam at the various pulse durations.



*Figure 12. Isotherm Rings and Drill Hole Size (140 mW)  
at pulse duration of:  
1 ms (upper left)  
3 ms (bottom)  
5 ms (upper right).  
The longer pulses are potentially damaging.*