



Applications of Infrared Lasers in ART

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ZILOS-tk® and LYKOS®

- **ZILOS-tk**

- Legacy laser system
- Launched 2002
- Patented design – only self-contained laser objective combination
- First laser to receive FDA clearance for LAH (2004)

- **LYKOS**

- New design introduced in 2011
- Improved optics and resolution
- Long working distance
- RED-i and multi-pulse standard



LYKOS DTS®

- **LYKOS DTS**
 - Introduced in 2019
 - Moveable laser
 - Computer controlled accuracy
 - Ease of use
 - Precise targeting features
 - Automated calibration
 - Built-in quality control reporting



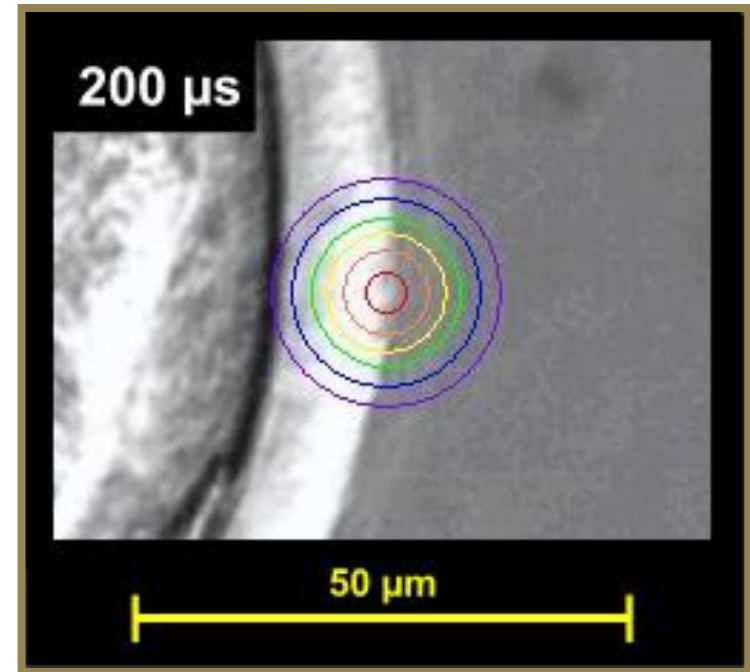
Clinical Laser Overview

- Laser integrated into custom 40x objective
- Functions in visible and infrared wavelengths
- Locked laser alignment
- Patented Isotherm Rings for embryo safety
- High portability
- Easy installation and setup
- Compatible with fluorescence



Laser Function

- Short laser beam pulses delete and breach intra- and extra-cellular membranes
- Laser beam focal diameter of 3.5 – 4.5 μm affords precise control to specific target
- Adjustable pulse length determines size of ablated area to 3.5 – 4.5 μm
- 1460 nm +/- 20 nm wavelength
- 300 mW max power
- 0 to 3000 μsec pulse length (duration), typically 200 – 600 μsec
- Isotherm Rings: Color-coded, turn viewing on or off (except laser)



Laser

140°C (hole size)

100°C

80°C

60°C

50°C

INFRARED BEAM is SAFE

Low energy photon [0.86 eV]

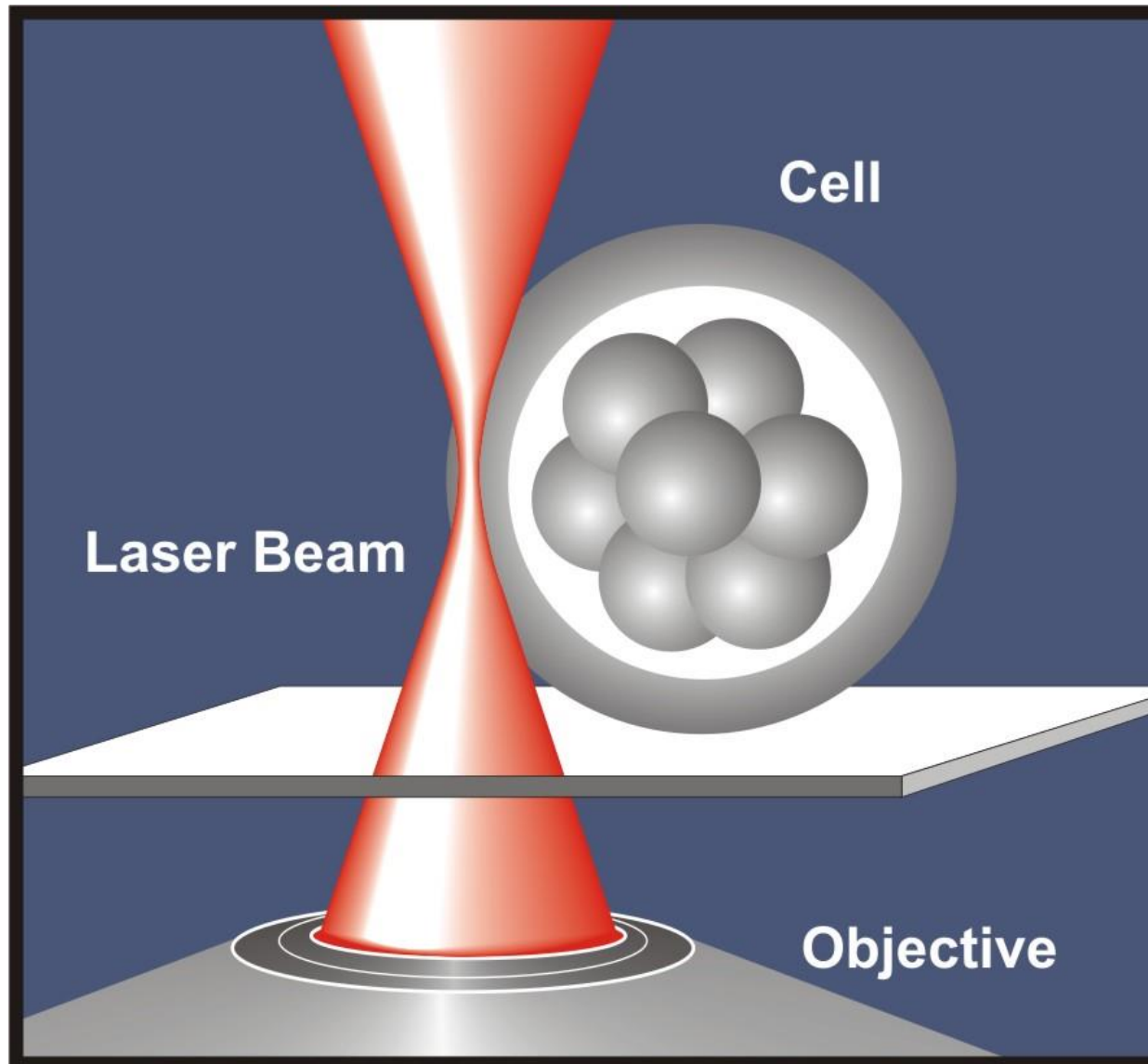
- **Cannot ionize molecules**
- **Cannot break molecular bonds**
- **Cannot cause chemical reactions**
- **Class I eye-safe**

ONLY EFFECT:

HEATS THE MEDIUM or ZP.

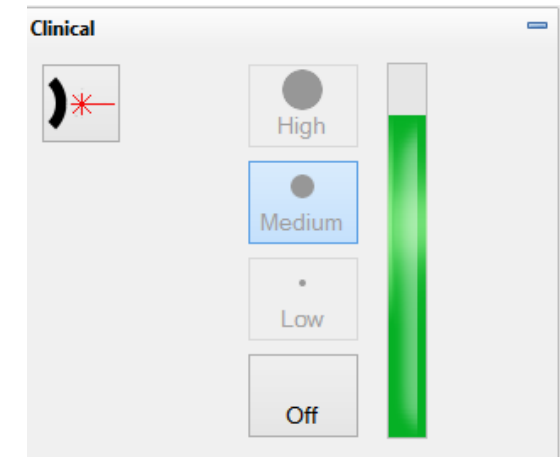
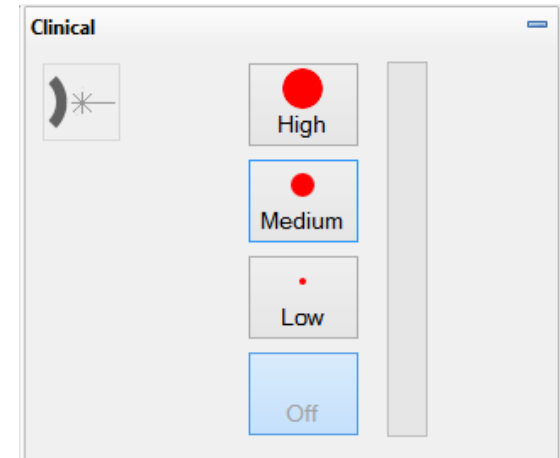
AVOIDS:

OVERHEATING EMBRYO



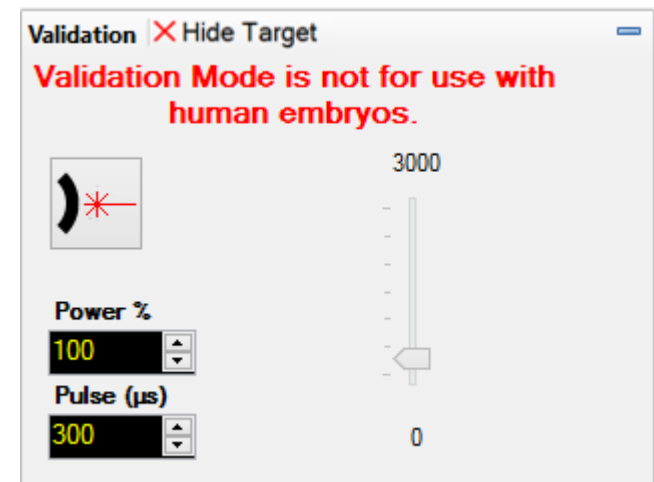
Clinical Mode

- **Three preset energies**
 - 100% power
 - Adjustable pulse length (μs)
 - Low: 1 - 200 μs
 - Medium: 200 – 600 μs
 - High: 600 – 3000 μs
- **Safety shutoff timer (automatic)**
 - Must fire laser within 15 seconds of activation



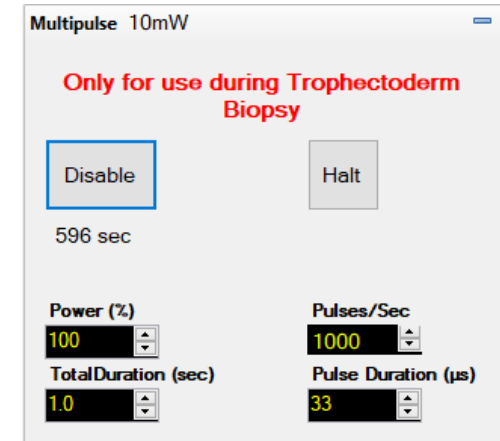
Validation Mode

- **Allows adjustment of both:**
 - Laser power: 1 to 100%
 - Laser pulse length: 1 to 3000 μ s
- **Used for target alignment**



Multi-Pulse: Independent Pulses

- Available on LYKOS laser
- Intense local heat for brief period
 - Select power, pulses/sec, pulse duration
 - 10 mW max mean limit
- Weakens intercellular bonds
- Separation of trophectoderm biopsy cells
- Reduces ex-incubator time: Automatic & rapid
- Increase effectiveness without increasing risk
- FDA 510(k) & MDD CE mark



Software Interface

HTLaser6

[Multipulse](#)
[Objectives](#)
[Reports](#)
[Ascii](#)

LaserBeam



100 um

[Settings](#)
[Help](#)

Multipulse 2.5mW

Only for use during Trophectoderm Biopsy

Disabled

Power (%)
 Pulses/Sec

Total Duration (sec)
 Pulse Duration (µs)

RED-i

☒ Enabled

Patient Info

Patient ID

First Name Last Name

Date Of Birth Physician

Embryo [Save](#)

Info Measurements Data Fields

Embryo

	um
1	118.4
2	118.1
3	121.6
4	122.7
5	121.9
Mean	120.5
SD	2.1

Number of Blastomeres

Images

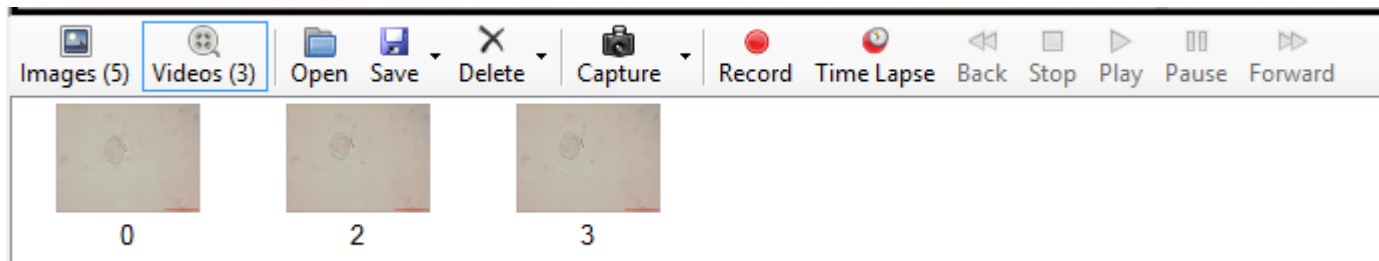
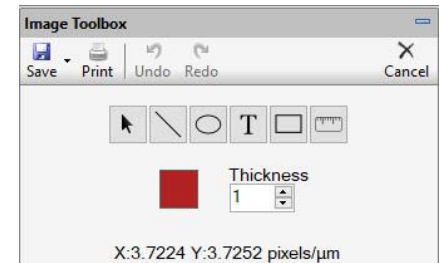
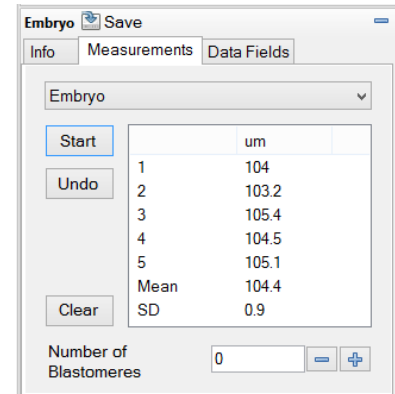
Images (5) Videos (1) Open Save Delete Capture Record Time Lapse Back Stop Play Pause Forward

0 1 2 3 4

ZTKMK3004 on COM4

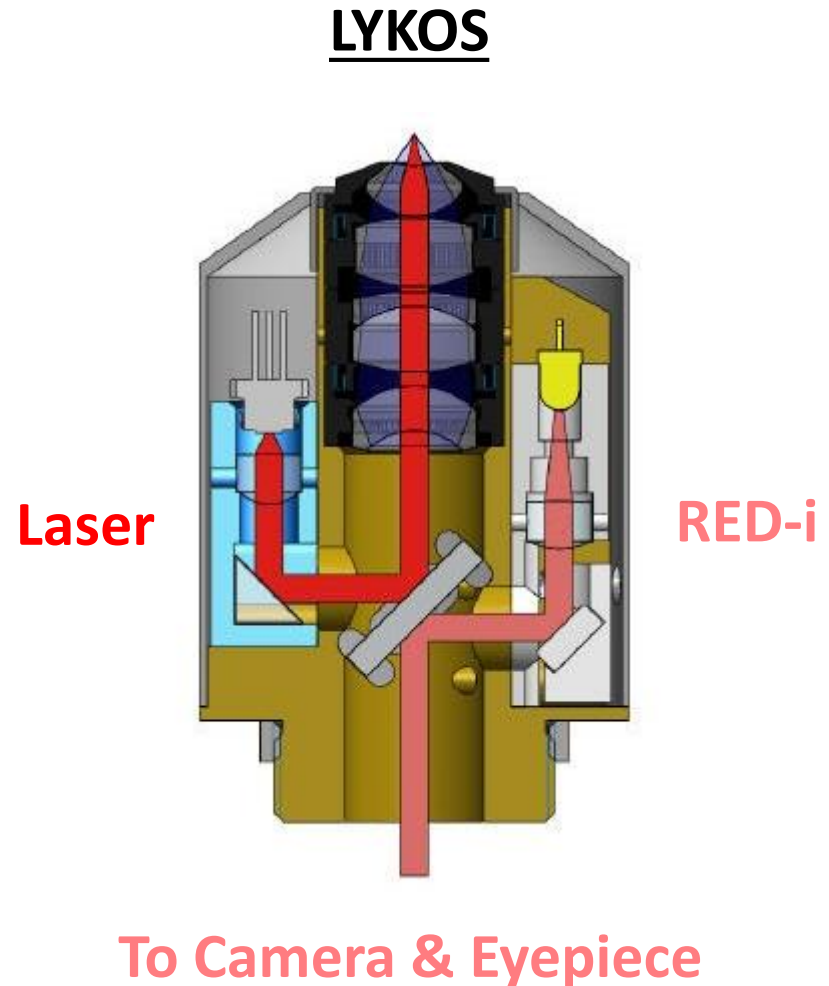
Additional Features

- Image capture
- Real time and time lapse video recording
- Measurement Toolbox: measurements saved to report
- Image Toolbox: Add freehand text, graphics and measurements
- Auto-labeling

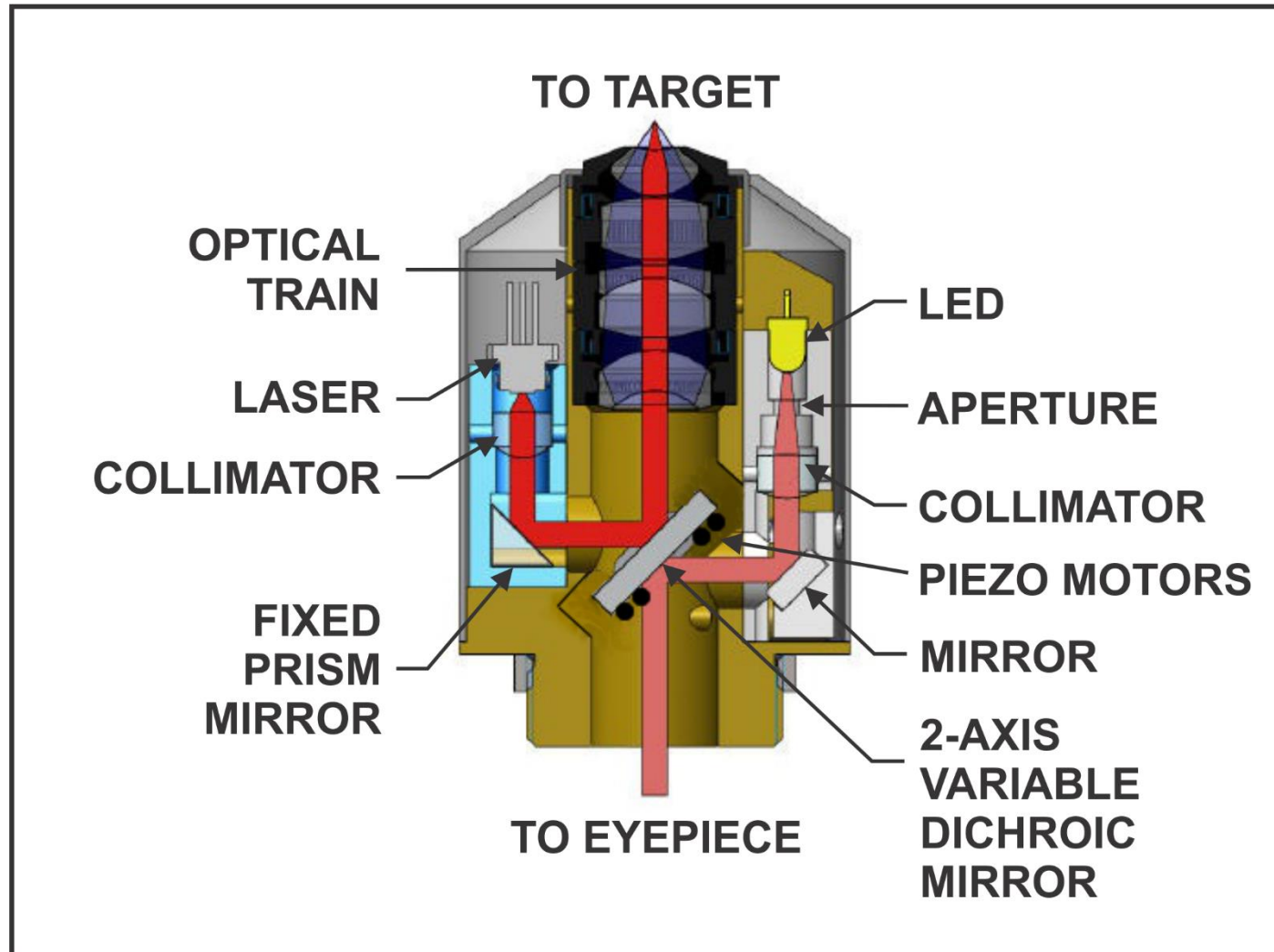


RED-i Target Locator

- The RED-i speeds workflow by allowing you to position the embryo under the laser beam without looking at the monitor
- Red LED indicator spot visible through microscope eyepieces
- Adjustable brightness level
- Always remains in focus
- Laser beam is NOT transmitted through eyepieces - SAFE for your eyes



LYKOS with DTS: Movable Laser



LYKOS with DTS Software

HTLaser6_Mike

Clinical New Objective Reports Ascii

ENGINEERING BUILD 1.12 Wed 04/10/2019 13:33:04.31 Settings Help

LaserBeam



Clinical Hide Target

13s

Hole Size μm

High 14

Medium 11

Low 8

☒ DTS Enabled

Multipoint

X Clear Path

RED-I

☒ Enabled

Patient Info Edit Delete

Patient ID 45076-33

First Name Charles Last Name Dawson

Date Of Birth 11/11/1980 Physician Dr. Jason Bloom

Embryo Save

Info Measurements Data Fields

Embryo ID 2019-2-14-03

Retrieval Date Thursday, February 14, 2019

Fertilization Method ICSI

Cycle Number 2 Embryos In Cycle 3

Note

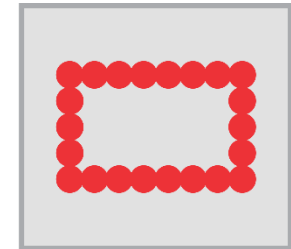
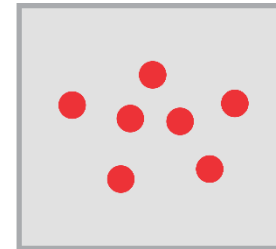
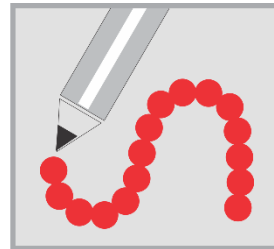
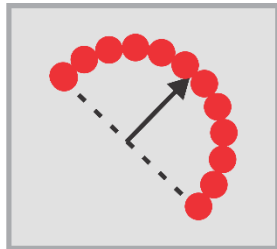
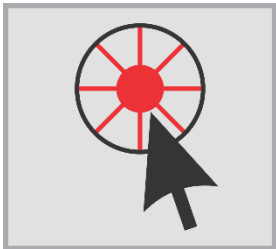
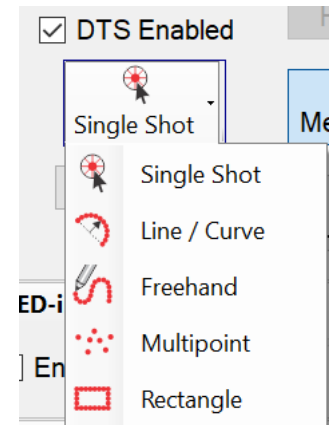
Images 1 2 3 4

ZTKMK3004 on COM11 DTS Connected - L6_MK1_005 on COM12

Objective Calibration X:4.02 Y:4.01

LYKOS with DTS: Laser Path Options

- Single Shot: Click any single point on the screen and a single laser pulse will be applied to that exact point.
- Line / Curve: Draw a straight line of any length and then select and drag to create a curve.
- Freehand: Any freeform path may be drawn by clicking and dragging the mouse pointer.
- Multipoint: Click up to 10 non-connected locations to apply laser. Each point may have a different laser setting.
- Rectangle: Click and drag to draw a rectangle of any proportion.





Initialization (Mapping)



Verification

AIM ACCURACY

LYKOS DTS INITIALIZATION & VERIFICATION QC (IVQC) History



Last Initialization:

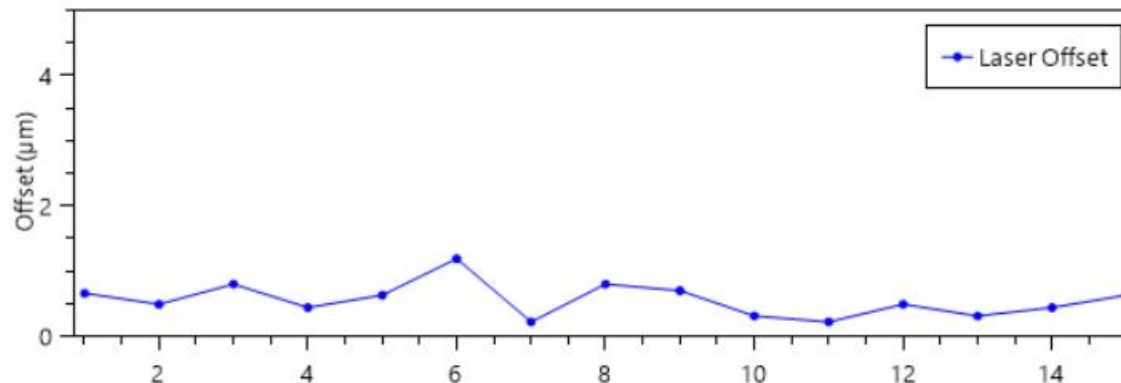
Tuesday, August 6, 2019 1:11:27 PM



Most Recent Verification:

Tuesday, August 6, 2019 1:36:30 PM

Offset Type	Mean Offset (μm)	Std. Dev (μm)	Max Offset (μm)	Status Pass / Fail
Laser	0.56	0.25	1.19	Pass
RED-i				





Multipoint



Freehand

Clinical Laser Applications

- **Assisted Hatching** (FDA cleared)
- **Blastomere Biopsy** (FDA cleared)
- **Trophectoderm Biopsy** (FDA cleared)
- **Polar Body Biopsy**
- **Blastocoele Collapse**

Assisted Hatching



Assisted Conception Unit,
Glasgow Nuffield Hospital
(Stephen Harbottle)

Blastomere Biopsy



Michael Tucker, PhD,
Shady Grove Fertility
Reproductive Science Center

Trophectoderm Biopsy



Georgia Kokkali, PhD
Genesis Athens Clinic

Polar Body Biopsy



Blastocoele Collapse



Yong Soo Hur
Kangwon National University
School of Medicine

Benefits of LYKOS®

Feature	LYKOS	Others
Laser components self-contained w/in objective	✓	X
Portability and ability to move between microscopes	✓	X
Highest image resolution	✓	X
Ability to parfocalize laser with other objectives	✓	X
Demagnification of image for embryo biopsy	✓	X
RED-i, safe for embryos and used during laser application	✓	?
Isotherm Rings based on actual pulse time and power	✓	X
Calibration upon each use	✓	X
Ability to position laser rather than positioning embryo	✓	✓
No need for pausing workflow to position laser for use	✓	✓
Low risk of unwanted movement	✓	X
FDA clearance for embryo biopsy and multi-pulse	✓	X

Some Applications

- **Laser assisted hatching**
- **Laser assisted biopsy**
 - Polar body
 - Blastomere
 - Trophectoderm
- **Blastocoele collapse for vitrification**
- **Post-warming AH**

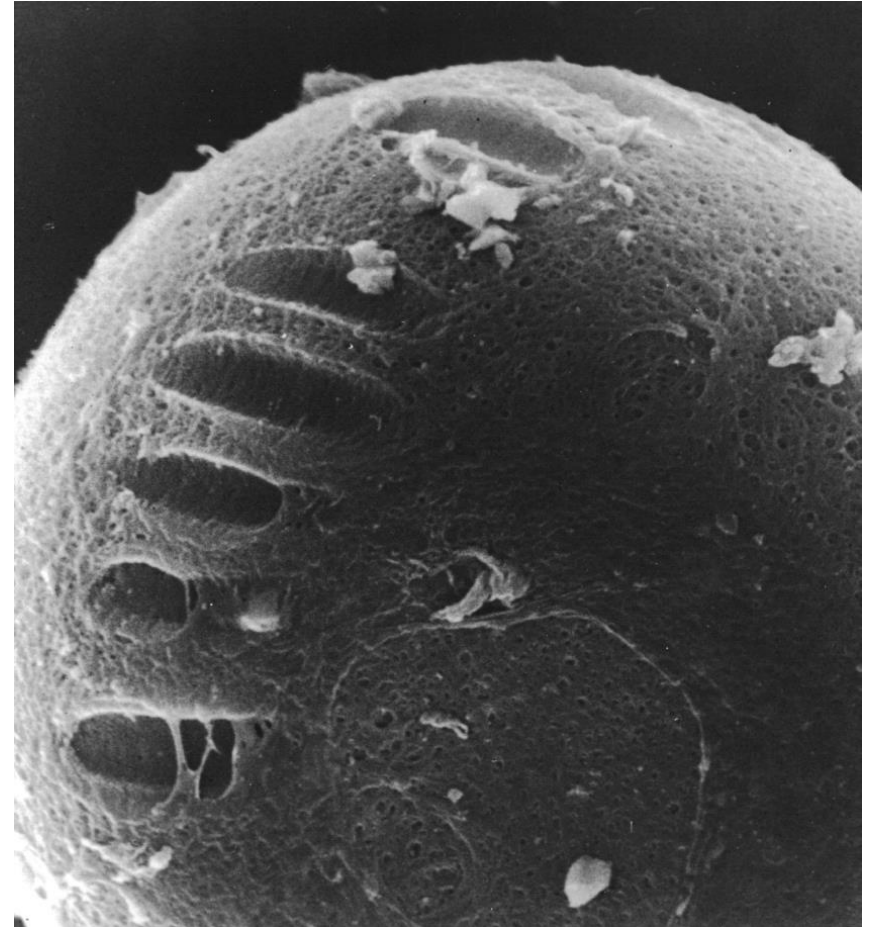


Photo courtesy Dr. Jerome Conia

Laser Assisted Hatching

- **Objectives of AH in the IVF Lab**
 - For non biopsied fresh or frozen transfers – to thin or completely remove the zona pellucida to allow hatching of the blastocyst embryo prior to implantation
 - AH can take place on Day 2 or Day 3 cleavage embryos or on Day 5 blastocyst embryos
 - Laser AH has been shown to increase clinical pregnancy and implantation rates
 - **Laser-assisted hatching increases pregnancy and implantation rates in cryopreserved embryos that were allowed to cleave in vitro after thawing: a prospective randomized study.** [Balaban B¹](#), [Urman B](#), [Yakin K](#), [Isiklar A](#). [Hum Reprod](#). 2006 Aug;21(8):2136-40. Epub 2006 Apr 13.

Laser Assisted Hatching

- **THE DEBATE: When should you AH your embryos for biopsy?**
 - Day 3 – Cleavage stage
 - Day 5 – Blastocyst stage

Laser Assisted Hatching - Day 3

PROS

- Takes place while embryos are out for Day 3 grading anyway
- Makes a nice opening in the zona and allows the trophectoderm to breach
- Keeps the zona thick – easier to hold onto during trophectoderm biopsy
- Makes the biopsy process faster – grab the breaching tissue, cut it off with the laser

CONS

- You have to hatch ALL your Day 3 embryos as you can not predict which one will become a blastocyst.
- May allow blastocyst to completely hatch out of the zona prematurely
- May allow some embryos to stick to one another via the breaching trophectoderm cells

Laser Assisted Hatching - Day 5

PROS

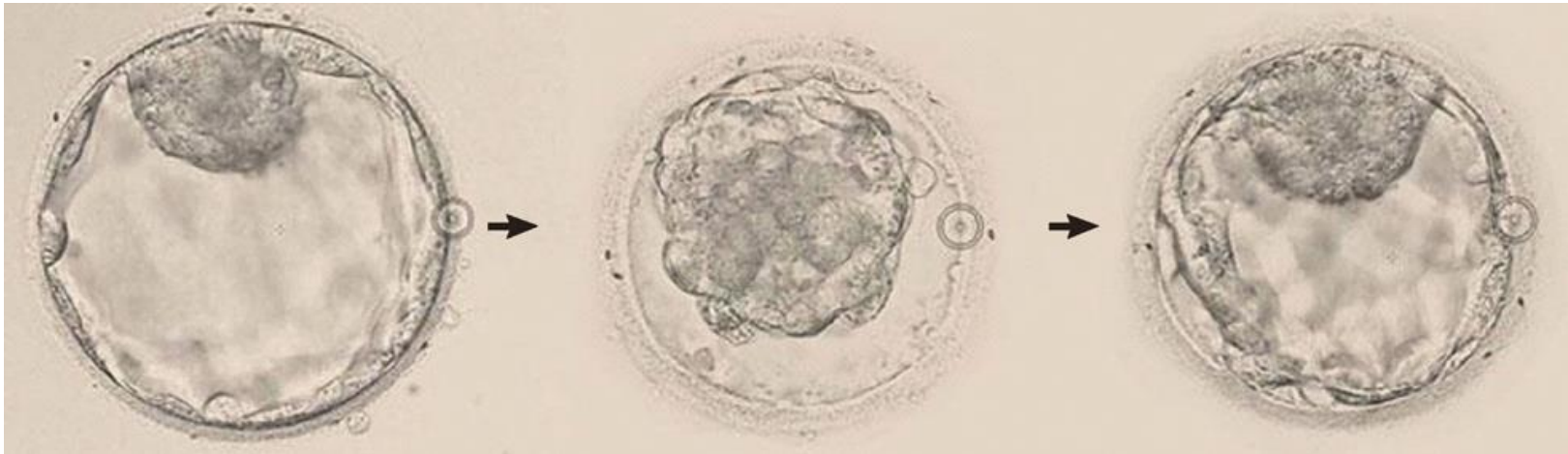
- Only AH the embryos that make good blastocyst and are ready for biopsy – less time out of the incubator on Day 3
- Decreased chance of embryos sticking to one another in culture – zona intact until time of biopsy

CONS

- Very thin zona – easier to pull out entire embryo during biopsy
- If conventional insemination was performed – increased chance of sperm contamination of your sample piece
- Makes the biopsy process a little bit longer for each embryo

Blastocoele Collapse for Vitricification

- Using the laser, the zona pellucida of a blastocyst stage embryo is opened, causing it to collapse.



- The collapsed blastocyst is then vitrified (quick frozen) by plunging into liquid nitrogen.

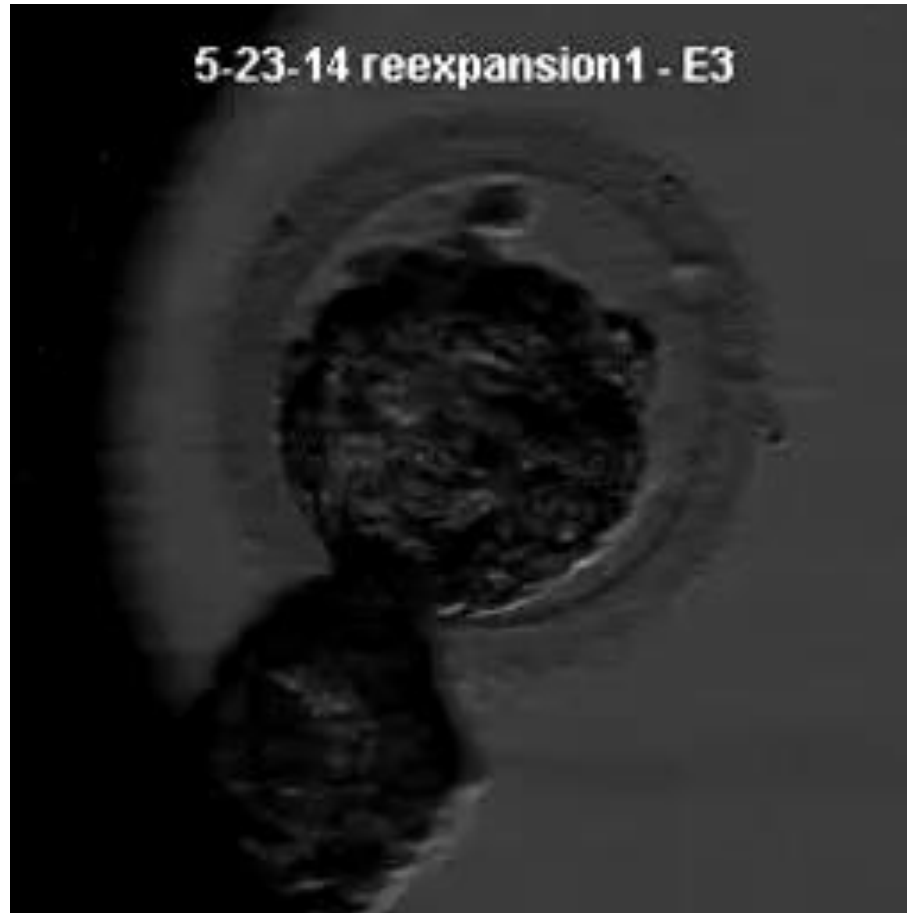
Benefits of Blastocoele Collapse for Vitrification

- Higher survival rates can be obtained by vitrification of blastocoele-collapsed blastocysts
- Vitrification causes less cell apoptosis in both mouse and human blastocysts compared to slow freezing.
- Vitrification of blastocysts after blastocoele collapse by single laser pulse supports a higher survival rate and less DNA apoptosis
- When collapsed blastocysts are vitrified, the equilibration time is fixed: stable and reproducible vitrification rate
 - **Comparison of DNA Apoptosis in Mouse and Human Blastocysts After Vitrification and Slow Freezing.** Lifei Li, Xuehong Zhang, Lihui Zhao, Xuefeng Xia and Weihua Wan. Mol. Reprod. Dev., 2012.

Benefits of Post-Warming AH

- What happens to the zona after thawing/warming?
- Recently, it has been shown through time lapse imaging that some embryos can remain trapped in the zona pellucida after thawing or warming in frozen embryo transfers
 - **Timing of Blastocyst Hatching after Vitrification and Warming: Impact on Clinical Pregnancy Rate.** S. Vaccari, PhD and J. Conaghan, PhD Fert Steril Vol 103: 2, Supp, Pg. e7–e8

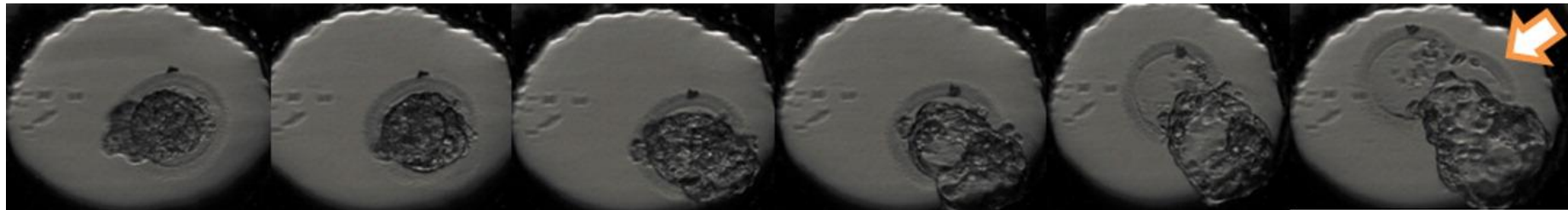
Benefits of Post-Warming AH



- Failure of a frozen biopsied embryo to hatch out of zona
- Nice figure 8, but biopsy opening is too small for embryo to escape
- Larger hold allows for complete hatching – increased chance of implantation and pregnancy

Benefits of Post-Warming AH

- Warmed embryo with large ZP opening at time 0, 1h, 2h, 3h and 4 hours respectively. Last panel shows detail of ZP “flap” (white arrow) that opened to allow escape.
 - **Timing of Blastocyst Hatching after Vitrification and Warming: Impact on Clinical Pregnancy Rate.** S. Vaccari, PhD and J. Conaghan, PhD Fert Steril Volume 103, Issue 2, Supplement, Pages e7–e8



Benefits of Post-Warming AH



Biggest and Best Labs Use HT lasers



Thank you!

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