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The Electroporation Experts

CELL TRANSFECTION & CELL FUSION
PRODUCTS

BTX-Division of Harvard Apparatus

ECM® 2001 Plus

Hybrimune™

Cell Fusion Theory

The formation of a single hybrid cell containing the nuclei and cytoplasm from different cells

- General Approach
  - Bring cells together so they touch
  - Compress the cells to increase surface area
  - Disrupt cell membrane
  - Provide a growth environment

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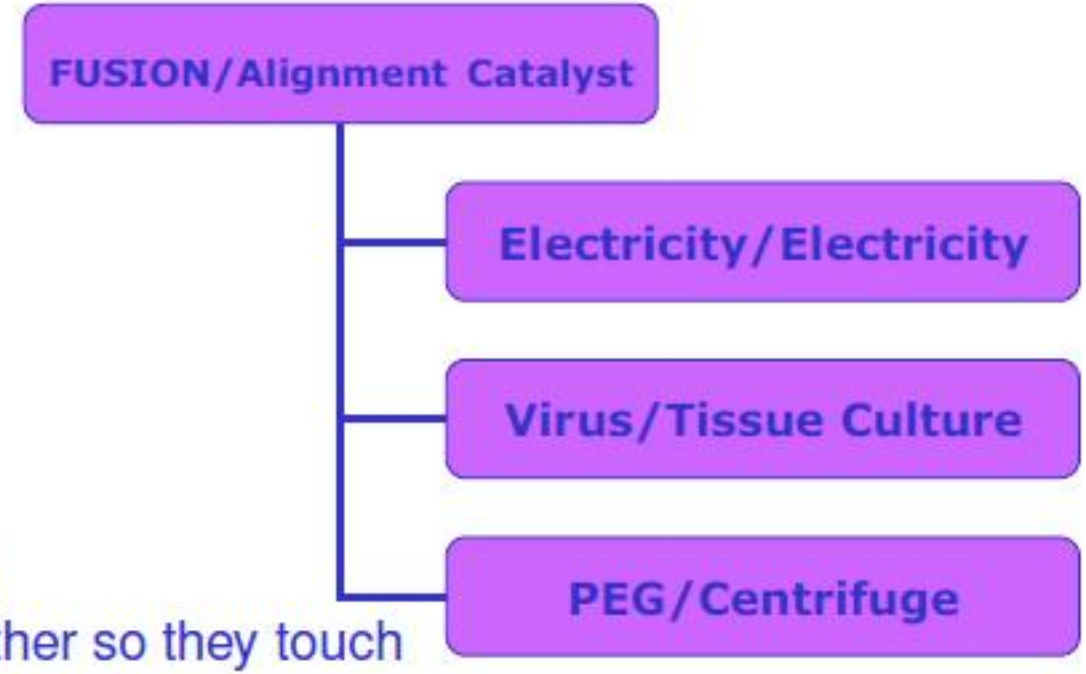
Cell Fusion Methods



General Approach

1. Bring cells together so they touch
2. Disrupt cell membrane
3. Provide a growth environment

PEG vs. Electrofusion

PEG-Mediated Fusion	Electrofusion
Peroxide build up contributes to cell cytotoxicity.	Not cytotoxic; Physical method
Lower fusion efficiencies	Hybrid yields are up to 10-fold over PEG-mediated fusion[8]
$>10^8$ cells are required for PEG-mediated fusion[5]	Fewer B cells required $<10^7$
Not reproducible: Too many variables (size & shape of the pellet; stirring method; technique varies from person to person)[5,6,7]	Optimized and reproducible protocols

Hybrid yields generated by Electrofusion are 10-fold greater than PEG

Experiment Number	Antigen Specific Clones	
	E-Fusion	PEG
1	20	0
2	10	0
3	400	23
4	151	21
Mean	145	11

Four different transgenic mice expressing Abs to human Ag were used to compare the efficiency of E-Fusion to PEG fusion.

Source: M. Coccia, Ph.D., Platform Development Group at Medarex, Inc., Milpitas, CA

Electrofusion resulted in 9X more Ag^+ reactive hybridomas relative to PEG fusion

- **8-fold more clones were obtained using electrofusion vs. PEG (542 vs 58)**
- **9-fold more hybrids tested positive to the specific antigen tested. Compared to those fused with PEG.(91 vs 12)**

		E-fusion			PEG-fusion	
Experiment	Ag	#γ,κ	#Ag,γ		#γ,κ	#Ag,γ
1	TT	336	96		ND	ND
2	TT	170	40		ND	ND
3	TT	208	20		0	0
4	TT	1400	10		150	0
5	TT	<1100	<400		83	23
6	TT	582	151		69	21
7	Ag #1	456	65		8	1
8	Ag #2	ND	168		ND	18
9*	Ag #3	493	101		128	56
10	Ag #4	71	0		0	0
11	Ag #5	323	0		47	0
12	Ag #5	246	0		36	0
Average		**542**	**91**		**58**	**12**
Difference (fold)		9	8			

*: scoring criteria different 0.5 vs 0.3, and all Ag,γ were mouse

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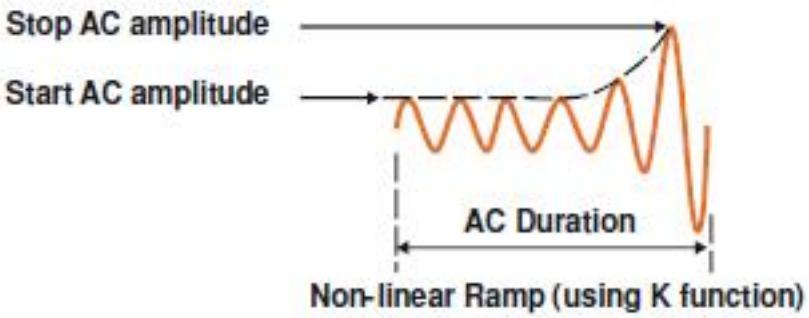
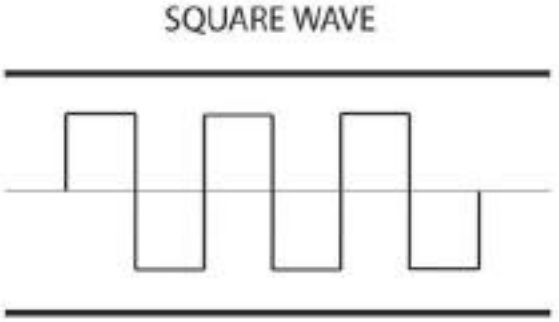
Electrofusion Mechanism

Electrofusion joins the membranes of neighboring cells by the application of a pulsed electrical field. The properties of two waveforms are used:



Oscillating AC Waveform



DC Square Waveform

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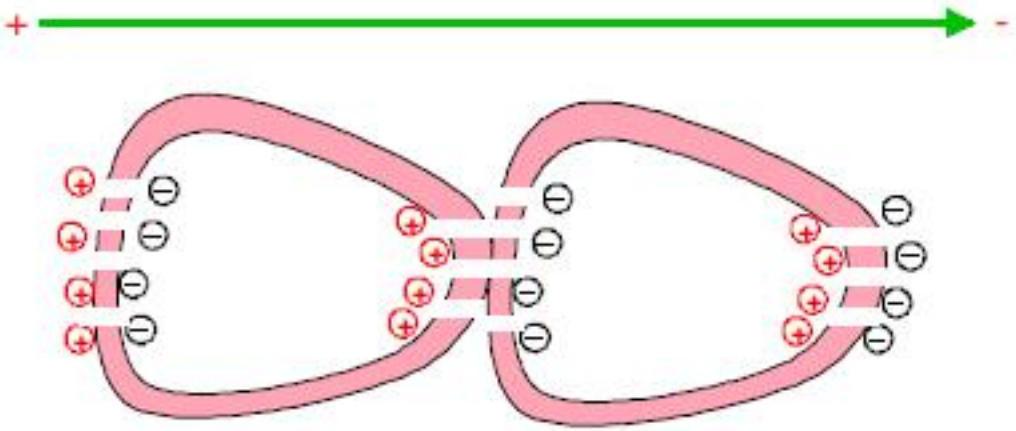
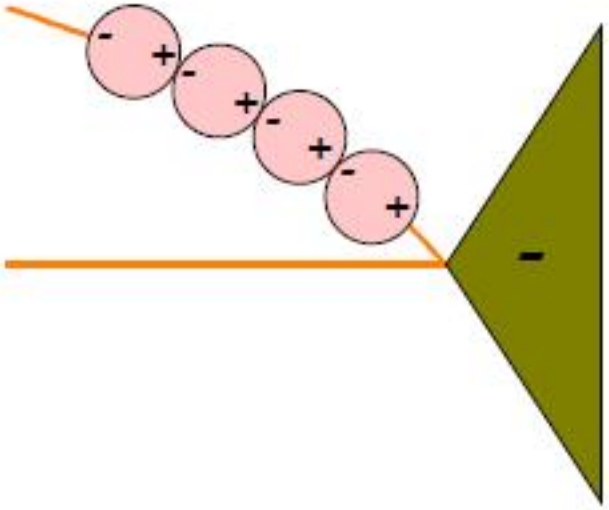
Fusion Pulse Disrupt Membrane

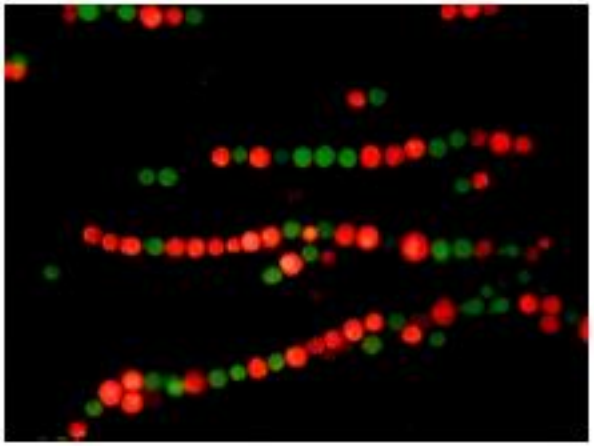
Apply a brief but intense Electric Field to form temporary pathways in cell membrane



Step 1-Pre-Pulse AC Cell Alignment

As cells move toward a common point the dipoles attract





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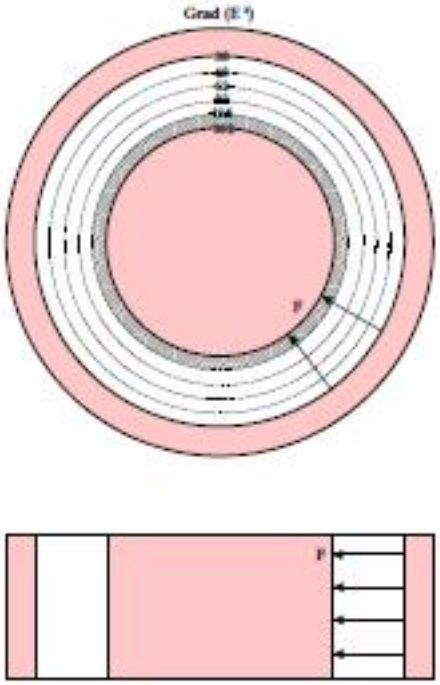
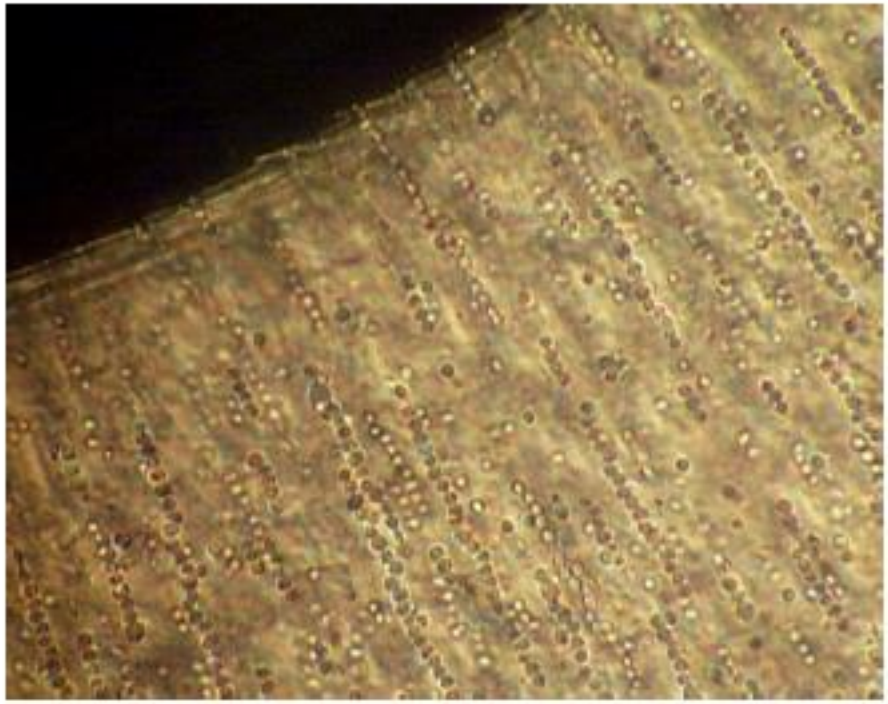
Coaxial Electrode (v^2/mm^3)





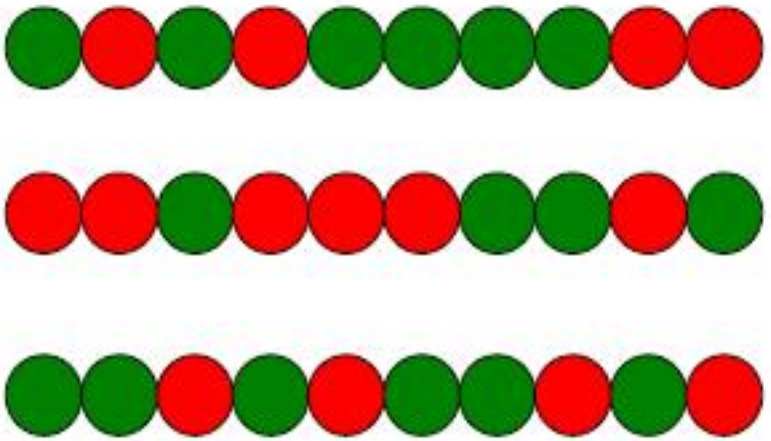
Adjacent Cell Probability

XY = 25%

YX = 25%

XX = 25%

YY = 25%



XY + YX = 50%

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Hybrimune® Hybridoma Production System

- Tri-Phasic Waveform Generator
- Computer controlled (not included)
  - Windows Interface
  - Record Keeping: Saves protocols/logs
- Large volume chambers for production
  - 2ml chamber optimization
  - 9ml chamber for production
- BTXpress Cytofusion Media
  - Specially formulated
  - GMP Compliant


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ECM 2001+ Enhanced Features

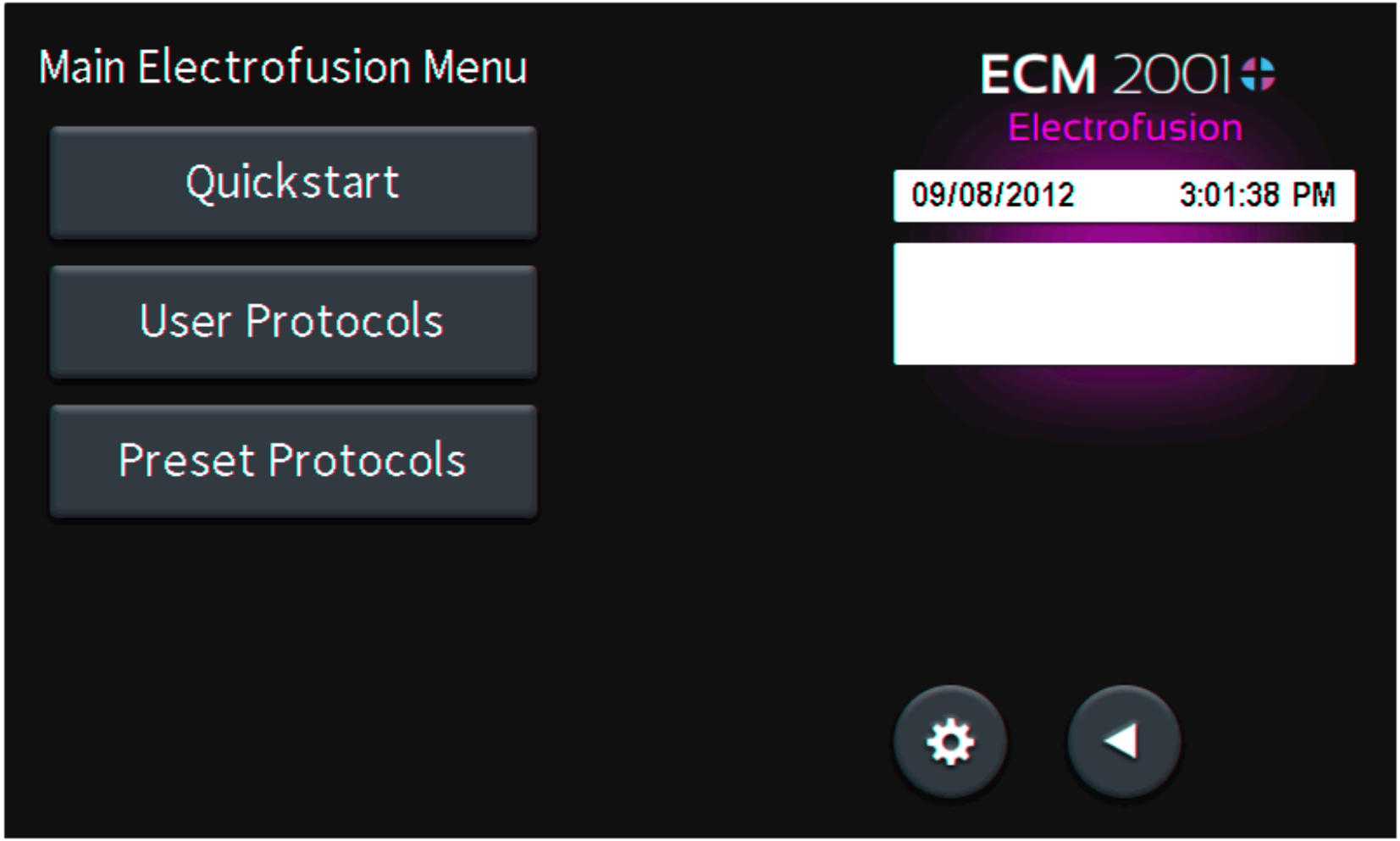
- Advanced protocol capabilities
  - Can combine up to 19 AC steps pre- and post- DC fusion step
- Enhanced AC sine waveform step programming capabilities
  - 0.2-2.0 MHz
  - 1-99 seconds
  - 5-75V, Linear ramping available
- New safety features, including pre-pulse sample resistance check, arc protection, and over current pulse abort.
- Large 7 inch touchscreen interface and integrated software within instrument
- Reduced footprint and weight
  - Length and width footprint space on bench matches current Gemini, however ECM 2001+ is taller in height.

ECM 2001+ Software Preview

Main Menu

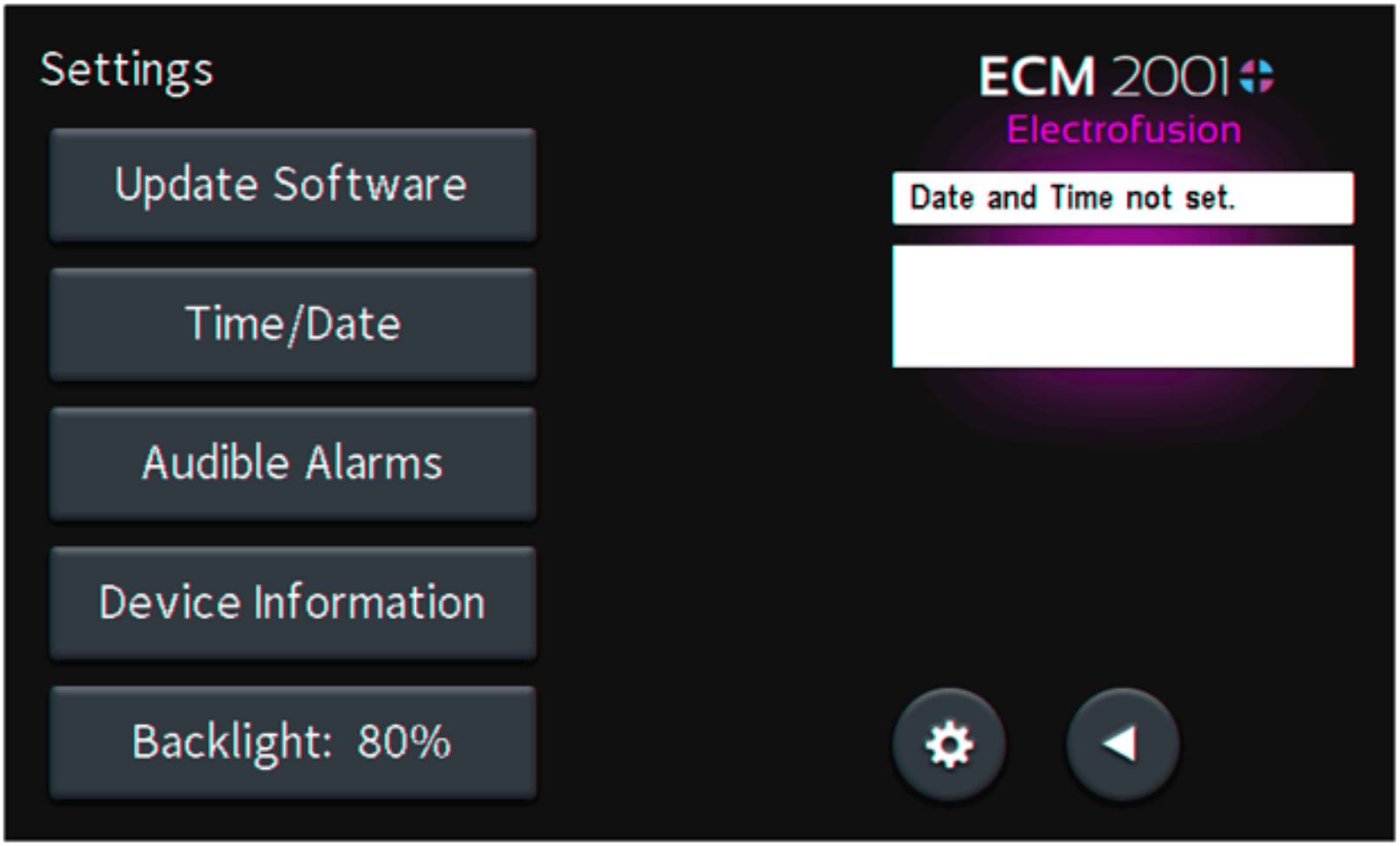
ECM 2001+ Software Preview

Electrofusion Main Menu



ECM 2001+ Software Preview

Device Settings Main Menu



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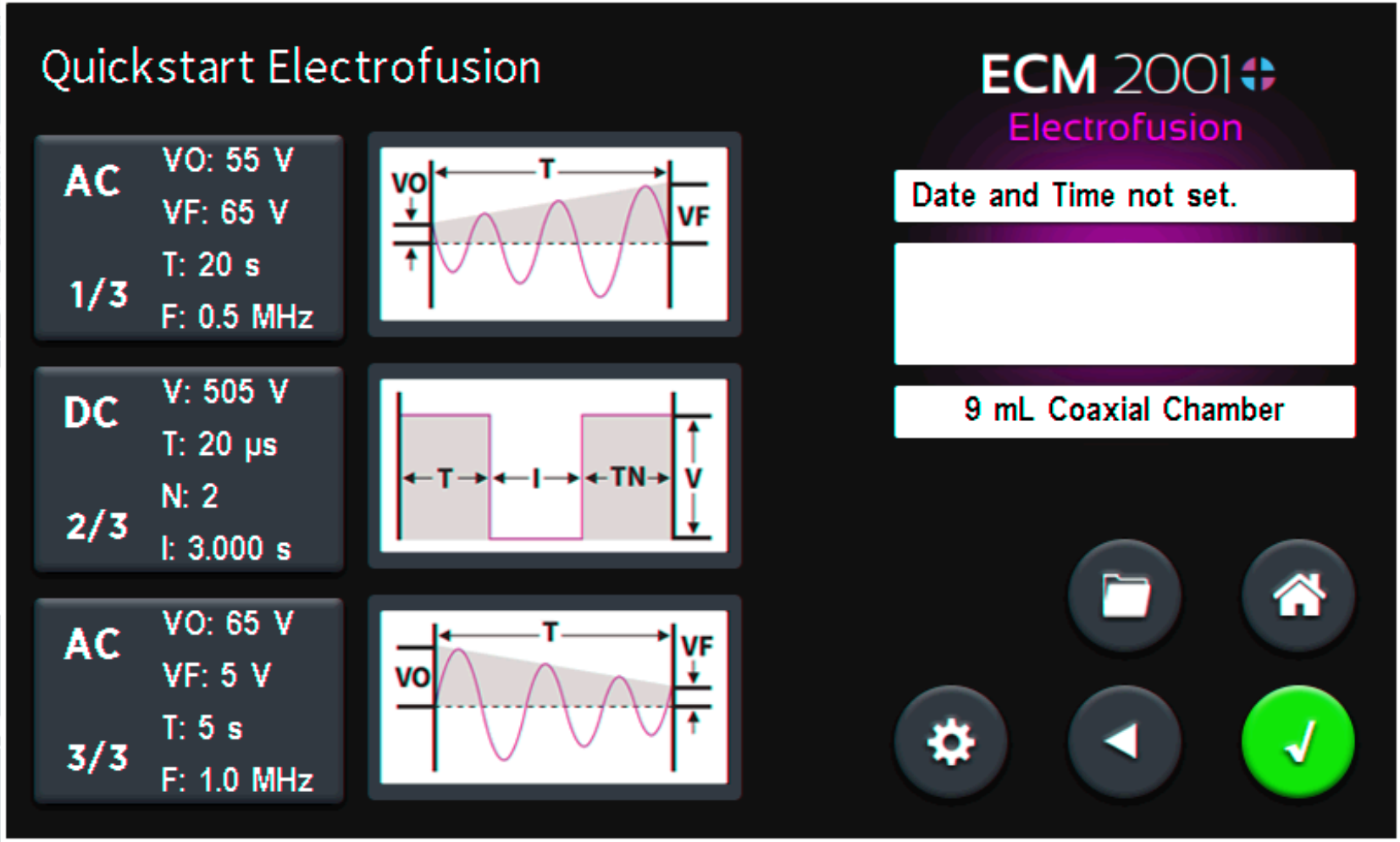

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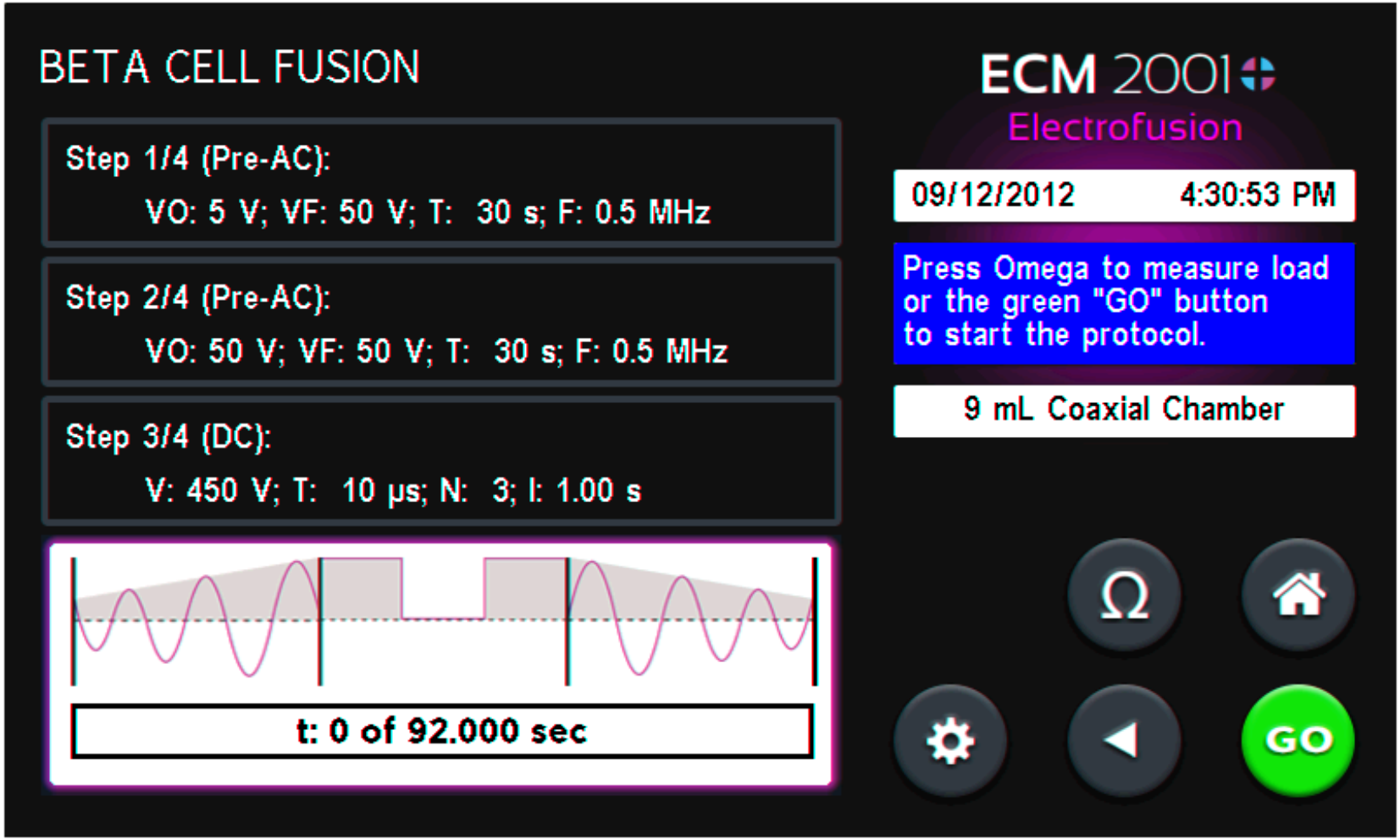

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Positioning ECM 2001+ vs. ECM 2001 and Hybrimune

	ECM 2001+	Hybrimune	ECM 2001
Target customers	Biotech/Industry/Pharma, some Academic	Biotech/Industry/Pharma	Academic/Teaching
Applications	Broad Electrofusion and Electroporation, Hybridoma Creation	Hybridoma Creation	Broad Electrofusion and Electroporation
Licensing Fee for Customer	No	Yes, **15K USD annually paid to Cellectis**	No
User Interface	Touchscreen	Connect to external PC (not included) running Hybrimune software	Manual buttons/knobs, Digital voltage displays
AC waveform	Sine wave, 0.2-2 MHz, 5-75 V peak, constant or linear ramping available, duration 0-99 s	Sine wave, 0.2-2 MHz, 5-75 V peak, constant, linear ramping, or exponential ramping available, duration 0-60 s	Proprietary waveform, 1.0 mHz fixed, 0-75 V_{rms} (Equivalent to ~106 V peak), constant, pre-fusion duration 0-99 sec, post-fusion duration 0-9 s, post-fusion voltage 1/10 of pre-fusion amplitude.
DC square waveform	5-3000V, 10 µs-999 ms	100-1000 V, duration 20-1000 ms	10-3000V, 10 µs-999 ms
Advanced programming	Yes; 1 DC step with up to 19 different AC steps total that may occur in any combination pre- and/or post- DC	Yes; Up to 10 different groups total, each group containing 0-1 each of 3 parts ordered AC/DC/AC	No; AC/DC/AC program with option of up to 9 additional sequential repeats of same fixed program
User protocols, Log file storage	Yes, >1000 on instrument SD card	Yes, stored on customer's PC	No
Preset protocols	Yes, >20	No	No


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Thank you