Cell-Detection Technique for Automated Patch Clamping

Candidate cells are identified automatically within one second.

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A unique and customizable machine-vision and image-data-processing technique has been developed for use in automated identification of cells that are optimal for patch clamping. [Patch clamping (in which patch electrodes are pressed against cell membranes) is an electrophysiological technique widely applied for the study of ion channels, and of membrane proteins that regulate the flow of ions across the membranes. Patch clamping is used in many biological research fields such as neurobiology, pharmacology, and molecular biology.] While there exist several hardware techniques for automated patch clamping of cells, very few of those techniques incorporate machine vision for locating cells that are ideal subjects for patch clamping. In contrast, the present technique is embodied in a machine-vision algorithm that, in practical application, enables the user to identify "good" and "bad" cells for patch clamping in an image captured by a charge-coupled-device (CCD) camera attached to a microscope, within a processing time of one second. Hence, the present technique can save time, thereby increasing efficiency and reducing cost.

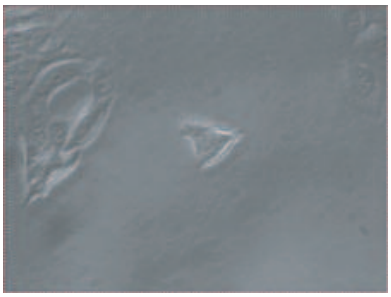
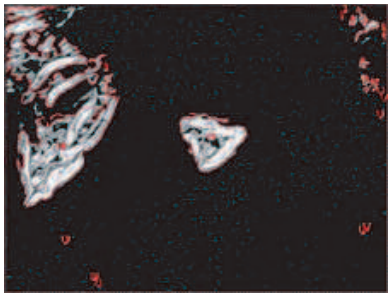
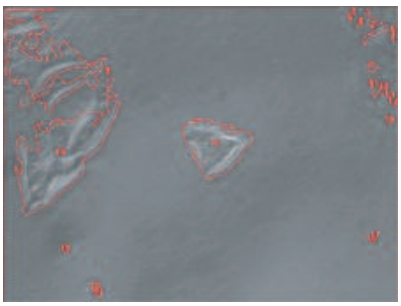


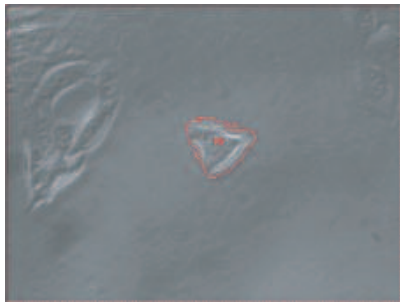
Image of Cells



Result of Applying "Find Particles" Technique to Image



Overlay of Identified Objects on Image of Cells



"Good" Candidate Cell for Patch Clamping

Number of Identified Object	x	y	Major-Axis Length	Minor-Axis Length	Area	Elongation	Roundness	Smoothness	Angle of Orientation	Thinness in Center	Partial Inclusion
1	72	89	191.8	126.7	12491	0.661	0.077	0.143	137	F	T
2	597	33	49.9	44.9	844	0.899	0.18	0.162	1.2	T	T
3	565	12	6.8	3.7	27	0.537	0.908	0.203	40.3	F	F
4	575	28	17.3	9.3	114	0.536	0.449	0.1	83.3	F	F
5	559	21	4	2.5	15	0.615	0.943	0.159	80.8	F	F
6	629	41	3.4	2.3	13	0.676	0.99	0.205	103.3	F	T
7	629	51	12	4	38	0.333	0.489	0.144	90	T	T
8	595	98	16	14	175	0.876	0.884	0.168	4.7	F	F
9	169	108	4	4	19	1	1.775	0.167	0	F	F
10	93	239	247.1	117.3	14243	0.475	0.093	0.139	44.7	T	F
11	599	129	35.8	14.6	277	0.409	0.323	0.185	99.4	F	F
12	614	132	22.1	5.5	121	0.25	0.361	0.161	76.5	F	F
13	623	146	5.4	1.9	15	0.346	0.619	0.135	74	F	F
14	630	158	3.2	1	7	0.302	0.752	0.188	74.5	F	T
15	342	223	122	88.4	6417	0.73	0.3	0.14	161	F	F
16	624	185	6.7	4.1	26	0.618	0.965	0.2	58.3	F	F
17	601	371	5.1	3.8	21	0.744	1.805	0.188	125.8	F	F
18	594	377	6.7	5	31	0.739	1.269	0.203	137.4	F	F
19	101	392	8	4.6	34	0.572	0.994	0.147	36	F	F
20	150	453	9.4	5	50	0.535	0.845	0.125	26.6	F	F
21	155	465	5.4	2.2	15	0.396	0.75	0.25	5.7	F	F

Note: Object 15 is the "best" candidate for patch clamping.

In this **Example of Application** of the technique described in the text, processing of an image of cells leads to identification of the approximately triangular object at the center as a "good" candidate cell for patch clamping. The table presents numerical results of analysis of 21 objects identified in the "find particles" stage of image-data processing.

The present technique involves the utilization of cell-feature metrics to accurately make decisions on the degree to which individual cells are "good" or "bad" candidates for patch clamping. These metrics include position coordinates (x,y) in the image plane, major-axis length, minor-axis length, area, elongation, roundness, smoothness, angle of orientation, and degree of inclusion in the field of view.

The present technique does not require any special hardware beyond commercially available, off-the-shelf patch-clamping hardware: A standard patch-clamping microscope system with an attached CCD camera, a personal computer with an image-data-processing board, and some experience in utilizing image-data-processing software are all that are needed. A cell image is first captured by the microscope CCD camera and image-data-pro-

cessing board, then the image data are analyzed by software that implements the present machine-vision technique. This analysis results in the identification of cells that are "good" candidates for patch clamping (see figure). Once a "good" cell is identified, a patch clamp can be effected by an automated patch-clamping apparatus or by a human operator.

This technique has been shown to enable reliable identification of "good" and "bad" candidate cells for patch clamping. The ultimate goal in further development of this technique is to combine artificial-intelligence processing with instrumentation and controls in order to produce a complete "turnkey" automated patch-clamping system capable of accurately and reliably patch clamping cells with a minimum intervention by a human operator. Moreover, this technique can be adapted to virtually any cellular-analysis procedure that includes repetitive operation of microscope hardware by a human.

This work was done by Mark McDowell of Glenn Research Center and Elizabeth Gray of Scientific Consulting, Inc. Further information is contained in a TSP (see page 1).

Inquiries concerning rights for the commercial use of this invention should be addressed to NASA Glenn Research Center, Innovative Partnerships Office, Attn: Steve Fedor, Mail Stop 4–8, 21000 Brookpark Road, Cleveland, Ohio 44135. Refer to LEW-17902-1.

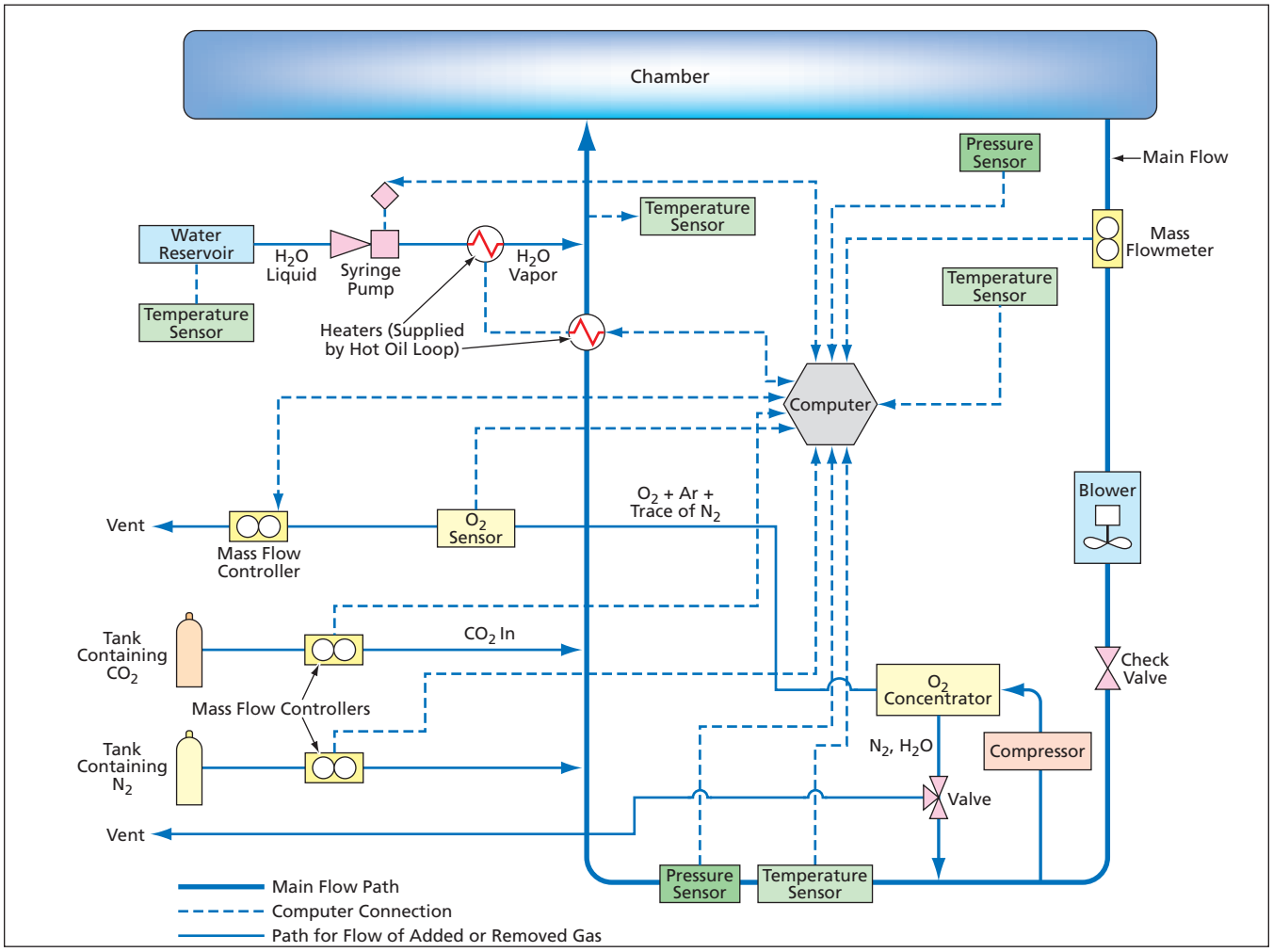
Redesigned Human Metabolic Simulator

Apparatus simulates atmospheric effects of human respiration.

Lyndon B. Johnson Space Center, Houston, Texas

A design has been formulated for a proposed improved version of an apparatus that simulates atmospheric effects of human respiration by introducing controlled amounts of carbon dioxide, water vapor, and heat into the air. Denoted a human metabolic simulator (HMS), the apparatus is used for testing life-support equipment when human test subjects are not available.

The prior version of the HMS, to be replaced, was designed to simulate the respiratory effects of as many as four persons. It exploits the catalytic combustion of methyl acetate, for which the respira-



The **Improved HMS** would remove O_2 while adding CO_2, H_2O, and heat in amounts chosen to simulate the respiratory effects of as many as eight humans at various levels of activity.