FUZZY CONTROL SYSTEM FOR A REMOTE FOCUSING MICROSCOPE

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ABSTRACT

Space Station Crew Health Care System procedures require the use of an on-board microscope whose slide images will be transmitted for analysis by ground-based microbiologists. Focusing of microscope slides is low on the list of crew priorities, so NASA is investigating the option of telerobotic focusing controlled by the microbiologist on the ground, using continuous video feedback. However, even at Space Station distances, the transmission time lag may disrupt the focusing process, severely limiting the number of slides that can be analyzed within a given bandwidth allocation. Substantial time could be saved if on-board automation could pre-focus each slide before transmission. The authors demonstrate the feasibility of on-board automatic focusing using a fuzzy logic rule-based system to bring the slide image into focus. The original prototype system was produced in under two months and at low cost.

Slide images are captured by a video camera, then digitized by gray-scale value. A software function calculates an index of "sharpness" based on gray-scale contrasts. The fuzzy logic rule-based system uses feedback to set the microscope's focusing control in an attempt to maximize sharpness.

The system as currently implemented performs satisfactorily in focusing a variety of slide types at magnification levels ranging from 10x to 1000x. Although feasibility has been demonstrated, the system's performance and usability could be improved substantially in four ways: by upgrading the quality and resolution of the video imaging system (including the use of full color); by empirically defining and calibrating the index of image sharpness; by letting the overall focusing strategy vary depending on user-specified parameters; and by fine-tuning the fuzzy rules, set definitions, and procedures used.

INTRODUCTION

The Space Station Crew Health Care System periodically collects microscope slides that must be analyzed by microbiologists on the ground within hours of collection. It would be desirable to minimize the time required for the crew to mount and focus microscope slides. However, simple telerobotic control by the microbiologist under visual feedback would entail several problems: first, a transmission delay of about two seconds in each direction will disrupt the focusing process, producing both slower and less satisfactory results than hands-on control would; second, because of the additional time required, the focusing process itself would tie up significant amounts of video downlink bandwidth, reducing the time available for the actual analysis; finally, the requirement to schedule slide mounting, focusing, image transmission, and analysis at the same time places tight constraints on all resources used, and may be highly inefficient and inflexible.

The system would be far more robust and efficient if the crew could collect the slide specimens and place the slides in a cassette or "jukebox", with no further crew attention required. An on-board robot could then mount each slide on the microscope, bring the slide into focus, and transmit one or more digitized still images to a computer on the ground. The microbiologists could then view and analyze the slides at their convenience. The resulting digitized images would require far less bandwidth to transmit than a continuous video image, yet each digital image could have better resolution and less noise than standard video.

Of course, in practice the microbiologist might still wish to be part of the focusing loop, either to concentrate on particular areas within a slide or to fine-tune focusing when the system's focus does not seem sharp enough. This could be accommodated by exception (recalling a slide that is still available in the "jukebox"), or

by allowing the microbiologist to preview each slide (in real time) and transmit a command that would modify or override the system's automatic focusing. Alternately (at greater cost but probably still less than full-video transmission), the system could transmit not one "focused" image but a series of images representing a range of focus settings.

The major technical challenge in this approach is the automatic focusing itself; mature technologies exist to handle the slide mounting, and to digitize, transmit, store, and display still slide images. This paper describes a demonstration prototype system that the authors developed at McDonnell Douglas Space Systems Company, with significant contributions by Apt Instruments, Incorporated (now Aptronix). The system demonstrates the feasibility of automatic focusing using fuzzy rule-based control and the possibility of producing similar control systems rapidly and inexpensively.

SYSTEM DEFINITION

Functions

Functionally, the system operates as a closed-loop discrete-time feedback system whose goal is to maximize the sharpness of a slide image by controlling the vertical position of the microscope stage (see Figure 1).

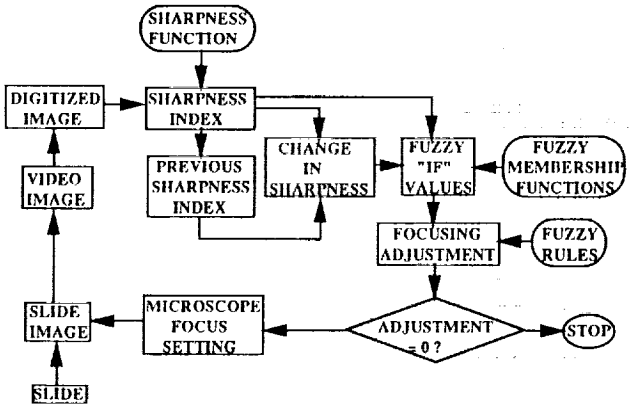


FIGURE 1

Slide images are captured by video and digitized. The system calculates a numerical scalar value representing the "sharpness" of any image from its digital record. This sharpness index and the difference between its current and previous values form the inputs to a system of fuzzy "if-then" rules that determines whether the maximum sharpness has been reached. If if has, the system stops and displays the resulting image. If the system has not reached its stopping point, the rule-based system specifies the amount and direction to adjust the microscope's focusing control. The result produces a new image, which starts the cycle again.

Hardware

Figure 2 shows the hardware used in the demonstration prototype system. This system was assembled to the authors' specifications by Apt Instruments, Inc., under contract to McDonnell Douglas Space Systems Company. An Olympus microscope with 10x, 100x, and 1000x magnification levels produces slide images that can be viewed directly, but are also transmitted to a Javelin black-and-white solid-state video camera. The video image is captured by a frame digitizer board on the 80386 personal computer. The digitized image can be displayed on a video monitor.

The 80386 personal computer performs the computations necessary to interact with the user, to compute the sharpness index, to run the fuzzy logic inference engine, and to specify desired focusing commands. These commands, in digital form, are transmitted to a motor controller, which translates them into pulses that drive a stepper motor. This motor, permanently mounted to the microscope's focusing mechanism, adjusts the vertical position of the microscope's stage.

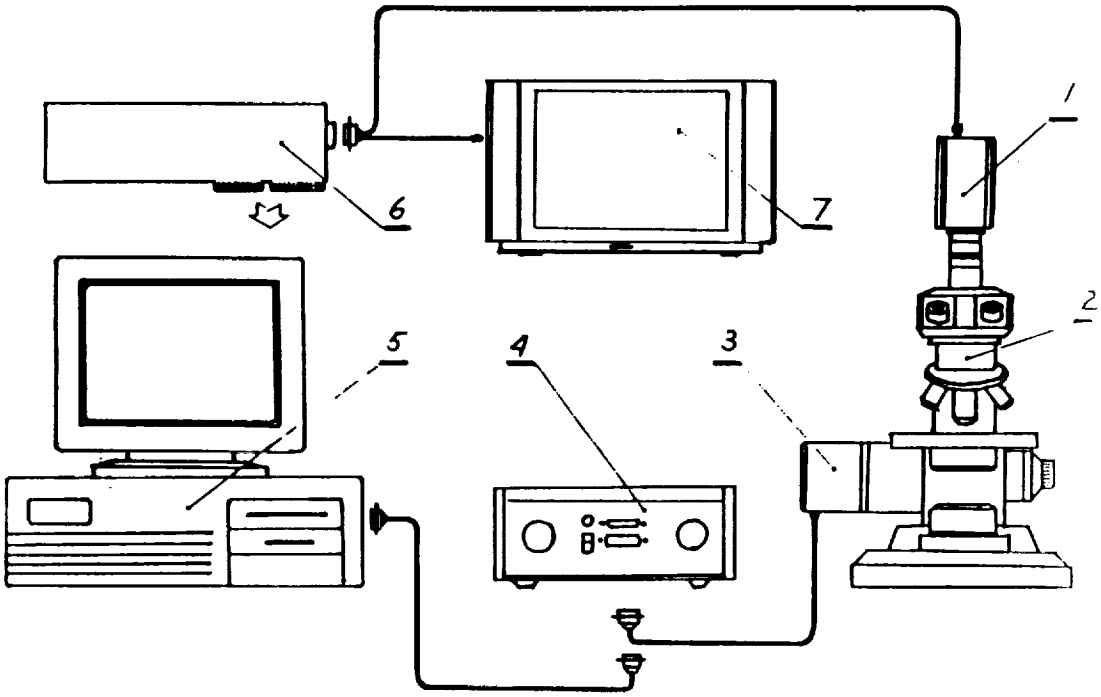
Software

Although much of the system's software covers routine functions such as system setup, input-output, etc., four components are worth describing in detail.

USER MENU

The user's interaction with the microscope system operates from a main menu of options. These are selected by pressing the function keys on the keyboard and are presented in logical sequence.

SET/RESET - This must be performed at system startup and whenever a new objective lens is selected. The user may also RESET the system at any time. The system prompts the user to select a level of magnification. (The current system requires the user to rotate the objective lenses manually; if the appropriate hardware were available it would take only minor software changes to control the lenses robotically.) Once a level of magnification has been selected, the user is prompted to set the absolute limits on the height of the microscope's slide stage. This prevents physical damage to the microscope or to the slide. (Again, a more sophisticated robotic system could perform this task automatically.)



SYSTEM HARDWARE CONFIGURATION

1. VIDEO CAMERA JE2362 2. MICROSCOPE BH-2 3. MOTOR M57-51 4. MACHINE CONTROLLER
5. IBM PERSONAL COMPUTER AT 6. FRAME GRABBER BOARD DT2855 7. MONITOR

FIGURE 2

FOCUS - FOCUS initiates the automatic focusing procedure, adjusting the height of the microscope stage until the sharpest image has been reached. When selected right after SET/RESET, FOCUS uses the entire digitized slide image as input to the computation of the sharpness index. When selected after AREA, it uses only the designated area(s).

MANUAL - In MANUAL operation mode lets the user control the microscope focus directly, using the computer keyboard. The "up" and "down" arrow keys move the stage up and down in fine gradations, while the "page up" and "page down" keys move in larger steps. After each keystroke, the stage is moved and the new image displayed.

AREA - This option permits the user to customize the focusing process by specifying up to three areas of the slide image. When one or more areas have been specified, portions of the picture outside such areas are disregarded when computing the sharpness index. This allows the user to exclude irrelevant objects, dust or scratches on the slide's cover slip, etc., which might otherwise interfere with the focusing calculations; it is also valuable at high magnifications, when the slide image may have objects that overlap at different depths or lie at an angle to the focal plane. Areas are selected by using the computer's mouse to draw an outline which is superimposed on the displayed digital image. Users also have the option to rate the "importance" (impact on the sharpness index) of the areas selected, choosing 'very high', 'high', or 'medium'. Once AREA has been completed, the next step is to select FOCUS again; because the sharpness index is computed differently, the FOCUS routine will in general select a different stage position as the best focus.

COLLECT DATA - The user is prompted to specify a (rectangular) area of the displayed image, using the computer's mouse. The selected area of the digital image is saved as a data file on the computer. This data could be used to provide a digital image that can be annotated or enhanced off-line, to document system activity or performance, or to provide input to image analysis programs.

RETRIEVE/DISPLAY IMAGE - The user can view a previously saved image file on the video display.

MODE - The user may select 'continuous' or

'single step'. 'Continuous' mode, which focuses the image without further action on the user's part, is the system's normal modeof operation. In 'single step' mode, the system stops after each focusing adjustment, giving the user time to view the image, to return to MANUAL mode, or possibly to save the image using the COLLECT DATA option.

COMPUTATION OF THE SHARPNESS INDEX

At the foundation of the focusing system is the idea that an image (represented as a rectangular array of digital gray-scale pixels) can be evaluated by computing a scalar-valued "sharpness index." This concept is appealing because once a suitable sharpness function has been defined, the problem of focusing can be reduced to finding the highest value for a function of a single variable (the height of the microscope stage) whose value is restricted to a known interval. Although there are a number of subtleties, technical difficulties, and empirical issues still to be addressed, the current demonstration prototype uses a fairly simple general-purpose sharpness function with generally successful results. (Some of these issues, with possible alternate approaches, are described later in this paper.)

The program calculates sharpness in the following manner:

(1) Reduce computation by sampling only a small number of lines from the image. Currently, the program samples 7 bands of three adjacent lines each.

(2) Treat each of the bands in the sample as a set of overlapping 3x3-pixel squares. For each 3x3 square, represent the gray-scale values as *a* through *i*, in the following configuration:

a	*b*	*c*
d	*e*	*f*
g	*h*	*i*

(3) Compute horizontal contrast as $x_h = (c+2f+i) - (a+2d+g)$, and vertical contrast as $x_v = (a+2b+c) - (g+2h+i)$. Total squared contrast for each 3x3 square is computed as $x_{tot}^2 = x_h^2 + x_v^2$.

(4) Sum the total squared contrasts for all 3x3 squares in the sampled bands to arrive at the raw sharpness index.

Essentially, this formula is the sum of squared local gray-scale contrasts, computed on a sample of the total image. For scaling purposes, the program also computes a normalized sharpness index, assigning a value of 100 to the best-rated image that is scanned during the SET/RESET routine as the user specifies the highest and lowest possible stage positions. The rationale for the current method is that as a well-focused image is blurred, sharp local contrasts will turn into smoother gradients, thereby lowering the overall sum of squared local contrasts.

IMPLEMENTATION OF FOCUSING STRATEGY

The focusing strategy employed by the system is based on a set of fuzzy rules. At each step in the focusing process, the current value of the normalized sharpness index and the difference between the current and the previous values are inputs to a fuzzy inference process described below. The output of that process is a suggested magnitude and direction for adjusting the microscope. For certain values (corresponding to well-focused images that do not show significant improvement with further adjustment), the rule-based system will suggest a change of zero; at that point, the focusing process stops.

The rules themselves take the form "IF *Sharpness* IS <descriptor1> AND *Change_in_Sharpness* IS<descriptor2> THEN *Correction* SHOULD-BE <value>". The two descriptors are labels for fuzzy sets defined over the possible values for *Sharpness* or *Change_in_Sharpness*, respectively. The value is a signed number corresponding to the magnitude of the focusing adjustment suggested.

In the current system, the range of possible *Sharpness* values is mapped onto six overlapping fuzzy sets. These sets roughly correspond to linguistic labels such as "very poor", "poor", "medium", "moderately sharp", "sharp", and "very sharp". Each of these sets is simply a function that assigns an integer value from 0 to 127 to any *Sharpness* value. A set of membership functions is illustrated in Figure 3.

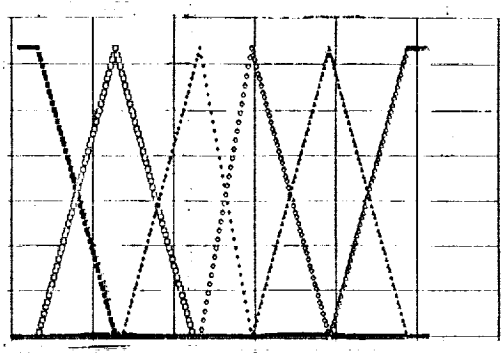


FIGURE 3

Similarly, *Change_in_Sharpness* has six membership functions. Thus, the system has a total of 36 rules.

Because the fuzzy values overlap, most of the time the rule input values (*Sharpness* and *Change_in_Sharpness*) match the "IF" conditions for two or more rules at levels greater than zero. When this occurs, the fuzzy inference technique used in this system computes a weight for each rule, which is the minimum of the two corresponding membership function values. All rules with nonzero weights are combined by taking a weighted average of the suggested outputs. For example, if Rule 1 had a weight of .25 and suggested an adjustment of +120 and Rule 2 had a weight of .75 and suggested an adjustment of +40, and all other rules had zero weight, the resulting suggested adjustment would be +60. This weighted-averaging technique assures smooth transition between adjacent sets of rules, an advantage compared to non-fuzzy rules which change actions abruptly as thresholds are crossed.

Special procedures are implemented to prevent undesirable situations. For example, if the image is not very sharp and does not appear to be improving, the rules may suggest a large step continuing in the same direction. But if such a step would exceed the limits defined in the SET/RESET process, the system adjusts the step to stay within limits. If this adjusted step still fails to improve, the system concludes that it has been looking in the wrong direction, returns to its starting point, and begins searching in the opposite direction.

SYSTEM PERFORMANCE

Operating on an 80386-based personal computer with an 80387 math coprocessor, the system generally reaches its "best focus" in a matter of seconds (corresponding to anywhere from 3 to about 25 focusing steps), as long as the slide contains sufficiently high contrast to generate meaningful values on the sharpness index. For very low-contrast slides, the system may fail to detect any objects and produce an error message during the SET/RESET procedure; slides that barely pass the initial test may cause the system to "search high and low" for any object, or oscillate between two settings without converging. It would be a simple matter to modify the code to detect this condition and terminate with an appropriate error message.

When the system reaches its "best focus", this means it has arrived at what it considers to be a sharp image. In some cases, this may differ from a human observer's judgment. Typically, it is possible to improve slightly on the sharpness of an automatically focused image by entering MANUAL mode and making fine adjustments. Part of this shortfall can be accounted for by the time-versus-quality tradeoff implicit in the focusing strategy and by the limited resolution of the microscope's motor; a more sensitive motor controller and a greater sensitivity to small changes in the sharpness value could increase the value of the sharpness function, but generally the magnitude of this improvement would be small.

The other component of the shortfall may be a mismatch between the sharpness index as computed by the system and the user's own concept of what constitutes a sharp image. Although preliminary investigations indicate that the current sharpness index performs adequately, more study is needed to determine if significant improvements can be obtained by defining the sharpness function differently. This may be particularly important at 1000x magnification, where greater shortfalls have been observed, possibly due to the more 3-dimensional nature of the 1000x images.

A side issue, arising during a pilot evaluation study with microbiologists as subjects, relates to the system's visual display quality. The standard-video, black-and-white images displayed on an ordinary video monitor were judged inadequate for practical used by microbiologists. Although full-color, high-resolution equipment would have solved this problem, it would have added significantly to the cost of the prototype demonstration system while shedding little additional light on the major technical issues addressed in the demonstration.

Even when the "best focus" agrees well with human observers' ratings, a second quality factor needs to be evaluated: the speed or efficiency with which the system reaches its focus. As already mentioned, the current system performs adequately, but occasionally it appears to make adjustments that a human observer would consider unusual. Improvements might be effected by modifying the rule set, fine-tuning the membership functions, or selecting an alternate method of reconciling overlapping rules.

To summarize, the current demonstration prototype has succeeded in its primary goal, which was to demonstrate the feasibility, low cost, and relative simplicity of a fuzzy logic approach to a complex control problem with space system applications. The system can focus most slides quickly and with adequate or better sharpness, although improvements on both factors should be possible.

TECHNICAL ISSUES

The purpose of the current prototype system

was to demonstrate the technical feasibility of fuzzy control for problems like microscope focusing. A working production version of such a system would require upgraded hardware and reprogramming (perhaps using a stand-alone microprocessor instead of a general-purpose computer). In addition to those changes, three technical issues need further study.

Defining the "sharpness" function

In the final analysis, even the most efficient system will fail to be effective if it is optimizing the wrong sharpness index. It is critical that the peak of the sharpness function correspond closely to the location human experts would select in manual mode. Beyond that constraint, it would be desirable if the sharpness function's shape were conducive to a fast and successful search.

The current method, a sum of squared local contrasts, works well for many images, but has some drawbacks. When there are two or more objects in different focal planes, for example, a larger object contains more pixels, and therefore contributes more to the sharpness index than a smaller one. Also, a "solid-colored" object exhibits local contrast only along its borders, while a striped, spotted, or other textured object shows contrast throughout its area and therefore contributes disproportionately to the sharpness index. Particularly at 1000x, some images also tend to produce optical artifacts (reflections or interference patterns) that appear as alternating bright and dark bands, generally around an object's edge; these bands are not real objects, but by contributing high contrast values to the sharpness index, they may throw the focusing process off. Finally, high-contrast extraneous objects such as dust particles, scratches on the slide, air bubbles, or water droplets tend to be located on the top and bottom of the slide and may "decoy" the focusing process away from the true targets.

The current method is only one of a vast family of possible "statistical" indices of sharpness. The computation of local contrast might use a 5x5 or larger region instead of a 3x3, or might weight the adjacent pixels differently. Instead of sum-of-squares, one could use the sum-of-absolute-values, the number or percent exceeding a given threshold, or the maximum contrast. Other statistical methods might use measues based on gray-scale or contrast-level entropy, statistical filters, or correlations.

These "statistical" sharpness measures share one drawback: they try to use the same index of sharpness for many different kinds of images in varying circumstances. By contrast, a human microbiologist knows what kind of slide is being mounted and has a pretty good idea of what to look for. For example, on one type of slide, a microbiologist may be interested only in a particular band of tissue, and in particular may need to know whether the band is a single unit or a set of adjacent components arranged end-to-end. If the crew member who collected each slide could simply indicate which type of slide it was, a sophisticated system could use expert rules to select the most appropriate statistical measure for each one. (A further extreme of sophistication might use advanced techniques in artificial intelligence, fuzzy logic, or neural nets to categorize the slide automatically and zero in on features of high interest; this would, however, go well beyond the original scope of this project.)

Strategies and tradeoffs for "optimization"

In defining an "optimal" search strategy, we implicitly make trade-off judgments about the system's desired performance. Once a sharpness measure has been chosen, each slide image could potentially generate a sharpness-versus-stage-position function defined over the range of possible stage positions. The problem is to arrive "sufficiently close" to the maximum value in an "acceptably short" time, with "high reliability". Although the system designer must work from an educated guess, the final evaluation of a system's performance must address all three issues, and must agree with the user community's subjective perceptions.

In the case of microscope focusing, there are three conditions that may complicate the focusing strategy:

(1) *Multiple local maxima.* Since it is not known at the outset what the maximum achievable sharpness index is, there is no guarantee that any local maximum is in fact the global maximum. However, if the sharpness function is reasonably well-behaved (free of tall spikes), it should suffice to start by taking coarse steps, then progress to finer adjustments.

(2) *Plateaus.* Sometimes, the sharpness index is fair to poor, but small to moderate-sized adjustments do not yield significant changes. This occurs often when the image is low-contrast, improperly lit, or far out-of-focus. The current system's approach is to continue in one direction until the sharpness index changes or until the upper or lower limit is reached; often this strikes the user as inefficient "blind searching". One possible approach might be to use a different kind of sharpness function until a certain quality of focus is reached, then transfer over to the "real" sharpness function. Along with this,

the rules might be modified (or an auxiliary set of rules developed) to take larger steps when on a plateau.

(3) "High-frequency" fluctuations. Particularly in the neighborhood of the maximum sharpness, very small adjustments seem to produce fairly large jumps in the calculated sharpness index. Some of this may be due to the resolution of the image digitizer, some to variations in the light source, and some to uncontrolled motion of the microscope or slide. Even at its finest adjustment, the stepper motor does not return the stage to its exact original position after one step up and one step back down. If these variations reflect the system's noise level, it would be advisable to modify the search strategy (or the sharpness function) to keep the system from getting caught in a prolonged series of very minor adjustments. If they represent the sharpness contributions of several values at distinct but close focal distances, there may be no universally satisfactory way to choose among them; the best solution may be to get the microbiologist into the decision loop, or to transmit several images instead of one. As in the case of the plateau, there might need to be a different set of rules for the case where sharpness is very good.

Fuzzy rule implementation

The current system uses one of several possible interpretations of fuzzy rules. In essence, it computes the degree to which the input data match the "IF" pattern for each rule and uses it as a weight for the numerical value specified by the "THEN" portion of the rule, taking a weighted average to reconcile when multiple conflicting rules "fire". This approach, which was developed by Apt Instruments, Inc., is conceptually simple and computationally efficient, but there is no evidence to date that compares its performance with other possible techniques. Therefore, it might be both instructive and prudent to compare several techniques to determine relative performance.

Another area for improvement would incorporate some sort of feedback or adaptive learning into what is now a static set of fuzzy rules and membership functions. This would most likely begin with an intuitive fuzzy model based on an expert's behavior or verbal instructions and, then, modify the rules and membership functions based on experience. One possible use might be to begin with a generic set of fuzzy rules and then develop a specialized version for each different type of slide.

CONCLUSION

The authors have demonstrated the feasibility of using a fuzzy rule-based system to control the automatic focusing of a microscope. Although several enhancements have been suggested, the current demonstration prototype illustrates that a very simple rule base and inference engine can be used to guide the focusing process successfully. The ability to produce working prototypes quickly and at low cost, coupled with the possibility of capturing the control process on small stand-alone processors, suggests that fuzzy rule-based systems may be an attractive way to implement automation in space.

ACKNOWLEDGMENTS

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