Paper:

Development of an Automated Microscope for Supporting Qualitative Asbestos Analysis by Dispersion Staining

Kuniaki Kawabata[*1], **Soichiro Morishita**[*2], **Hiroshi Takemura**[*3], **Kazuhiro Hotta**[*4], **Taketoshi Mishima**[*5], **Hajime Asama**[*2], **Hiroshi Mizoguchi**[*3], **and Haruhisa Takahashi**[*4]

[*1]Kawabata Intelligent System Research Unit, RIKEN
Hirosawa 2-1, Wako, Saitama 351-0198, Japan
[*2]RACE, The University of Tokyo
[*3]Faculty of Science and Technology, Tokyo University of Science
[*4]Department of Information and Communication Engineering, The University of Electro-Communications
[*5]Department of Information and Computer Science, Saitama University
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This paper introduces automated microscopic observation supporting qualitative asbestos analysis. Visual qualitative asbestos evaluation generally involves dispersion staining. Operators conventionally check and count asbestos fibers visually by microscope. We are developing automated microscopic observation to support qualitative asbestos analysis. The system images fibers by microscope and saves them automatically to a database. We introduce system concepts and performance using the prototype we developed.

Keywords: asbestos, microscopic observation, qualitative analysis, automation

1. Introduction

Asbestos, a naturally occurring mineral valued for its low cost, light weight, expandability, durability, heat and chemical resistance, soundproofing, and insulation, was once widely used in insulation, molding boards, fireproofing, etc., in the construction of housing, schools, and other buildings. Once it became known that inhaled asbestos fibers penetrated and adhered to lung tissue and, 20 to 40 years later, caused malignant mesothelioma, lung cancer, and other health problems, use of the material was restricted, and asbestos analysis became indispensable in determining whether currently used building materials contained enough asbestos to harm the health [1–3].

Asbestos analysis may be qualitative, determining whether a building material contains enough asbestos to damage health, or quantitative, determining a specimen's actual asbestos content. Qualitative analysis generally combines X-ray diffraction analysis and visual inspection. X-ray diffraction analysis has largely been automated, but visual inspection has not, lowering work efficiency due to the labor and time visual inspection requires. The recent increase in asbestos-related problems has concomitantly increased requests for asbestos analysis, making it imperative to raise analytical efficiency. A simple asbestos detection test [4] developed recently uses a specific protein combination to detect asbestos in the air, but detects only chrysotile and not other forms of asbestos. It is also not based on JIS standards.

We have developed a system to support dispersion staining, a type of qualitative analysis used to measure asbestos. We focus here on providing an overview of experimental functions and note that requirements thus far identified have been met. Specifically, the system supports visual inspection in dispersion staining by quickly and automatically recording and storing imaging specimens for later analysis.

2. Qualitative Asbestos Analysis

Qualitative analysis determines whether a specimen contains asbestos fibres primarily through combined X-ray diffraction analysis [5] and dispersion staining [6]. In X-ray diffraction analysis, a specimen is X-rayed and the X-ray diffraction peak obtained and compared to the unique asbestos diffraction peak. Dispersion staining, which is representative of visual inspection processes, enables observers to identify asbestos fibre color dispersion based on immersion and polarization.

Specifically, in dispersion staining [7]:

1. A specimen is observed under an objective lens at $\times 10$ magnification to see if fibers indicate dispersive colors. If so, the lens is switched to $\times 40$ magnification.
2. All particles in the visual field of an eyepiece providing $\times 40$ magnification (up to 1,000) are counted regardless of type, size, and shape. Particles may be any substance, but those showing unique asbestos coloring are recorded. JIS prescribes the unique dispersive asbestos colors listed in **Table 1**.
3. The above processing is done in three immersion liq-

Table 1. Dispersive asbestos fiber color.

Asbestos	Refractive index	Dispersion color
Chrysotile	1.550	Red purple ～ Blue
Amosite	1.680	Pink
	1.700	Blue
Crocidelite	1.680	Orange
	1.700	Blue

Table 2. Qualitative analysis decision criteria.

X-ray diffraction method / Dispersion staining method	detect unique diffraction peak	not detect unique diffraction peak
over 4 fibres in counted 3000 particles	proceed to quantitative analysis	proceed to quantitative analysis
below 4 fibres in counted 3000 particles	Re-analysis by Dispersion staining method	specimen not contains asbestos

uids having different refractive indexes to determine whether four or more asbestos fibre fibers have an aspect ratio of 3.0 or more in a total of 3,000 particles.

This result is compared to that of X-ray diffraction analysis to determine whether asbestos fibres are present (**Table 2**).

The greatest factor compromising dispersion staining work efficiency is the actual counting in visual inspection. Field interviews indicate that a single operator can analyze, at most, just 10 specimens a day.

In considering official dispersion staining procedures, [7] observation should include at least the following functions:

- Arbitrary specification of imaging range and location
- Polarizing plate angle specification and control
- Automatic image saving
- Synchronous computer control of these functions

Such functions are required because official procedures require that particles and asbestos fibres be counted after a visual field is selected at random, that dispersive color changes characteristic of dispersion staining be aggressively pursued, and that specifying which views include counted particles or asbestos fibres is difficult.

With these points in mind, we designed experimental automatic observation centering on a personal computer (**Figs. 1**–**2**, **Table 3**).

3. Automated Microscope for Dispersion Staining Support

In proposing technological development supporting analysis efficiency in dispersion staining, we focused on image collection – selecting a field of observation using a microscope – for evaluation complying with official procedure in time and labor.

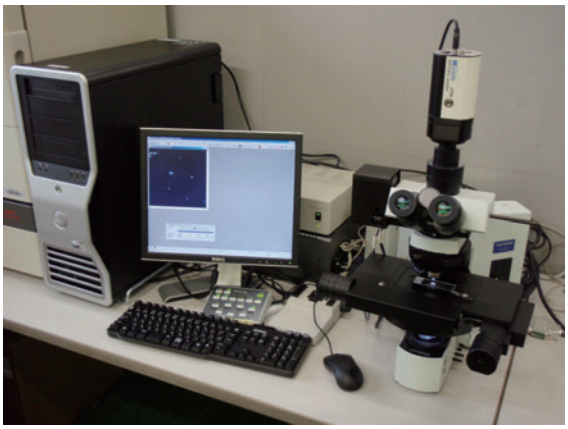


Fig. 1. Prototype.

Table 3. System component specifications.

Equipment/device	Specification
Computer-controllable type Phase Contrast Microscopy (OLYMPUS BX61N-33DPH)	object lens for phase contrast image (×10, ×40) object lens for dispersion color (×10, ×40)
Computer-controllable analyzer	Polarizing plate control precision 1° (max:0.01°)
Electric cooled color CCD camera (QImaging Retiga 4000R-C-Color)	1" interline CCD device resolution : 2048×2048 pixel size : 7.4×7.4um RGB resolution : 36bit IEEE1394 I/F
Computer-controllable XYZ stage (Prior Scientific ProScan H101)	travel range : 108×67mm control precision : ±1um

We targeted a system that imaged a specified observation area seamlessly. Systems now in use such as Magiscan [8, 9] and the Asbestos Fibers Automatic Counting System (AFACS) [10, 11] evaluate images focusing on airborne asbestos fibres, and are useful because, in addition to asbestos fibers, air specimens normally contain only ultrafine, mostly nonvisible particles. Inoue et al. [12] proposed automated asbestos fiber counting based on image edge information, again targeting airborne asbestos fibers and neglecting particles such as pulverized construction materials.

Building material specimens suspected of containing asbestos contain other substances roughly equivalent in size to asbestos fibres, so dispersion staining is used due to the relative ease in distinguishing asbestos fibres from other particles. This means that any new system for analyzing the asbestos content of building materials must consider dispersion staining requirements.

Because a phase contrast dispersive microscope is used in dispersion staining, we used System Microscope BX61 (Olympus Corporation, Japan). A source installed under the microscope illuminates the specimen to be observed, which is then recorded through the microscope lense by a CCD camera (Retiga 4000R, QImaging Corporation) (**Table 3**). Camera imaging settings are controlled by image processing library software (Image-Pro Plus, Media Cybernetics Inc.). Current design specifications require 36-

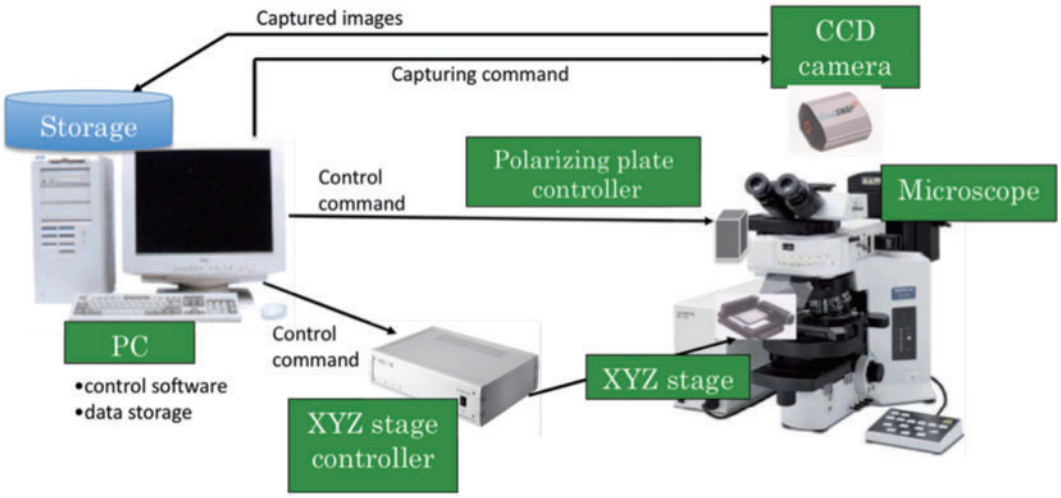


Fig. 2. System configuration.

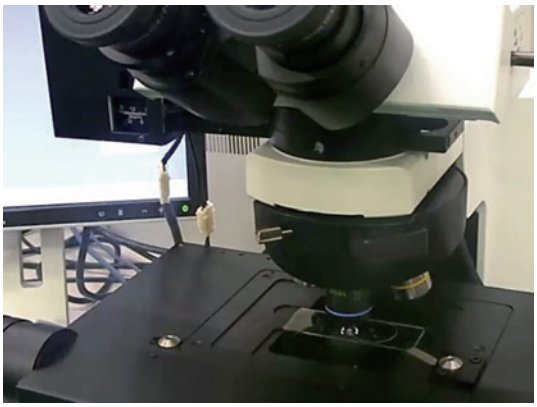


Fig. 3. XYZ stage and automated polarizing plate unit.

bit color (12 bits each for R, G, and B), 24-bit color (8 bits each for R, G, and B), 12-bit monochrome, and 8-bit monochrome images saved in Multi-TIFF format. Color information is required for dispersion staining (Resolution requirements, which are related to file size, will be set after further research and experiments.).

Imaging is done by shifting the XYZ stage, which holds the specimen, under the microscope's objective lens. The XYZ stage is automatically positioned (**Fig. 3**) at the mechanical origin, prior to imaging, and becomes the coordinate origin for later processes. To specify an observation area, operators set the stage's upper left and lower right ends via the microscope's joystick assisted by a graphical user interface (GUI), which determines the observation area from three points – the origin, the lower right end, and the upper left end. Focusing along a plane is also done by measuring the focus at each point and calculating an object imaging plane, although this is not usually required for ordinary specimens.

To control the polarizing plate (analyzer) angle (**Fig. 3**), the polarizing plate controller uses a stepping motor with an angular resolution of 0.01° operated via a software driver. Official procedures hold that dispersive colors are easily recognized if the polarizing plate is rotated 90° or more. When the practical angular resolution for observation is set to 1°, the possible range is from 0° to 180°.

Our system controls XYZ stage positioning, polarizing plate angle control, and the shutter for the CCD camera. Individual system components are connected to a PC (**Fig. 2**) and the system is controlled automatically and synchronously, automating image data collection for dispersion staining.

Experiments using the system are detailed in the sections that follow.

4. Imaging Experiment

We tested our experimental imaging system to check its operational utility. Official procedures require that a visual field be arbitrarily selected and particles and asbestos fibres counted. After considering precise particle and asbestos fibre counting in a selected visual field, we decided to image the entire observation area by shifting the microscope's local view. The specimen to be observed is placed under a 22×22 mm glass cover placed manually on a regular glass slide. For an effective imaging area 10×10 mm, 729 images are captured because a single imaging area observed by the microscope is 0.379×0.379 mm.

In imaging experiments, is shown in **Fig. 4**, the polarizing plate, set to 0°, 45°, and 90°, and the imaging shutter are controlled simultaneously. Internal monitor illumination is changing slightly. (The change in dispersion color of an asbestos fiber is shown at upper right.) As stated, one local image saved in 36-bit color multi-TIFF format is about 18.45 MB. Three polarization angle patterns imaged at each point in a 10×10 mm imaging area seamlessly thus require a file of 40.4 GB per sample.

To control the polarizing plate and camera shutter synchronously during imaging, the experimental system repeatedly sends a polarizing plate control command to the controller and receives a rotation end response after the image is captured in the camera exposure time specified. Theoretically, image capture requires 1 sec. and image data saving, stage movement, and polarizing plate rotation require 0.5 sec. Capturing images consisting of three

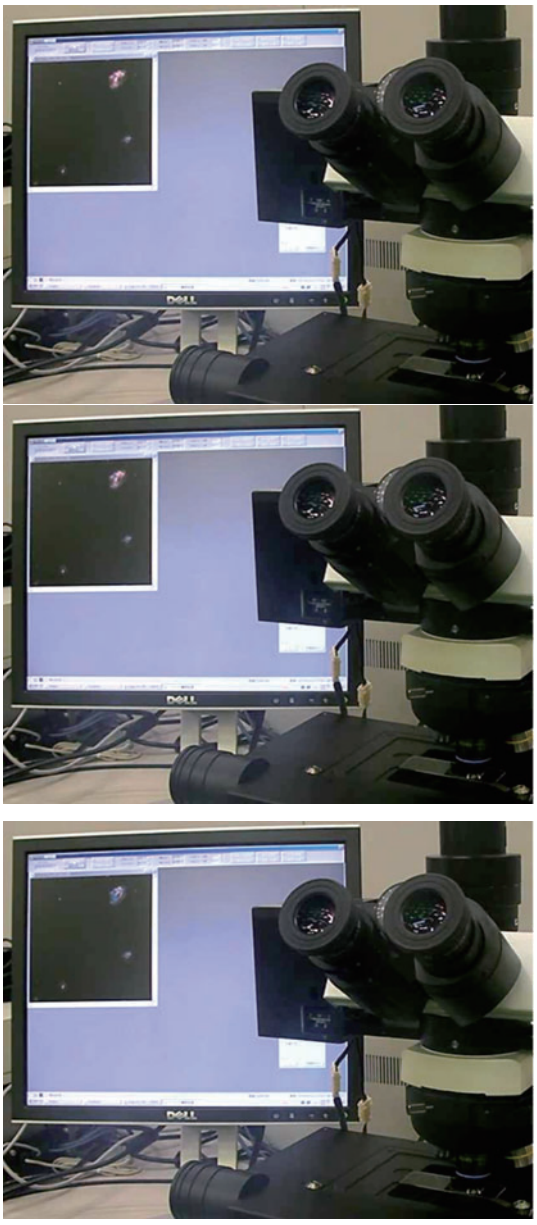
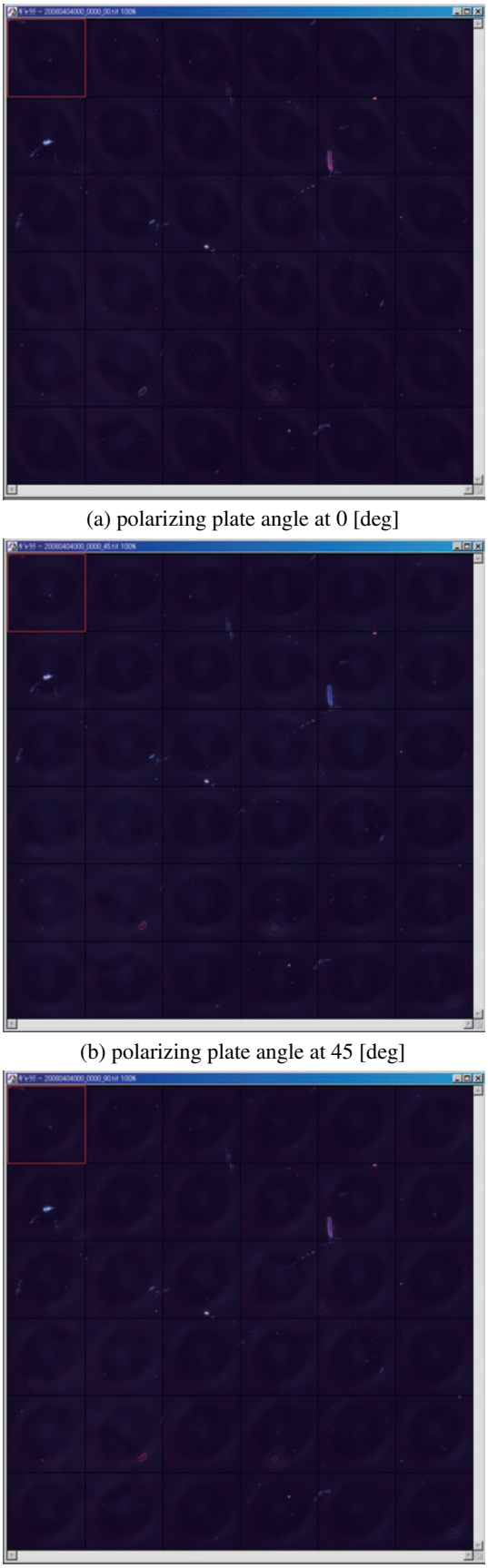


Fig. 4. Examples of image capture experiments.

polarization patterns from the 10×10 mm cover glass requires 4.5 sec. ×696 = 3,132 sec., or about 52 min.

Field interviews indicate that one operator can analyze a maximum of 10 specimens a day. Our system collects and analyzes microscopic image data at a level equivalent to that of experts. Operators can observe and judge microscopic images on a wide monitor, and the system lightens operator burden. Captured images are also stored and managed – something not done in conventional dispersion staining. Such evidence is important for analysis results.

Each captured image is digitally saved to HDD together with the specimen and positioning IDs. The system's GUI control enables operators to recall images, enlarge or reduce image display, and reproduce images in tile patterns based on the positioning ID (**Fig. 5**; polarizing plate angle: 0°, 45°, 90°. Red squares indicate a single captured



(a) polarizing plate angle at 0 [deg]

(b) polarizing plate angle at 45 [deg]

(c) polarizing plate angle at 90 [deg]

Fig. 5. Examples of images reproduced in a tile pattern based on positioning ID.

area 0.379×0.379 mm. Note the changed asbestos fiber dispersion color at upper right.)

As stated, we have focused on providing an overview of experimental functions, and requirements thus far identified have been met, i.e., the system supports visual dispersion staining inspection by quickly and automatically recording and storing imaging specimens for later analysis. We expect that further discussions with field workers will lead to more practical specifications in the future.

5. Conclusions

We have outlined the development of automatic observation supporting the use of dispersion staining, which is important to qualitatively analyzing asbestos particles in building materials. Based on information stated in official procedures, we created a system that automatically controls the polarizing plate angle, captures microscope images, and saves image data. At the least, the system substantially reduces labor required for imaging an entire observation area.

We are now working on automatic counting to combine asbestos fibre and particle detection techniques with image processing. [13 – 15]

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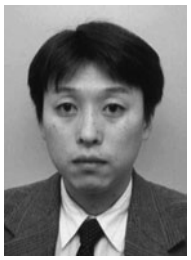

Name:
Kuniaki Kawabata

Affiliation:
Kawabata Intelligent System Research Unit, ASI, RIKEN (The Institute of Physical and Chemical Research)

Address:
2-1, Hirosawa, Wako, Saitama 351-0198, Japan

Brief Biographical History:

1997- Joined Biochemical Systems Lab. at RIKEN as a special postdoctoral researcher
2000- Joined Advanced Engineering Center at RIKEN as a research scientist.
2002- Joined to Distributed Adaptive Robotics Research Unit, RIKEN
2005-2007 Unit leader of Distributed Adaptive Robotics Research Unit, RIKEN
2007- Senior research scientist, RIKEN
2007- Unit leader of Kawabata Intelligent System Research Unit, RIKEN

Main Works:

• K. Kawabata, Y. Komori, H. Asama, and T. Mishima, "An Asbestos Fibres Detection Technique Utilizing Image Processing Based on Dispersion Colour," Particulate Science & Technology: An Int. Journal, 2009 (accepted).

Membership in Academic Societies:

• The Japan Society of Mechanical Engineers (JSME)
• The Robotics Society of Japan (RSJ)
• Society of Instrument and Control Engineers (SICE)
• The Institute of Electrical Engineers of Japan (IEEJ)
• Institute of Electrical and Electronics Engineers, Inc. (IEEE)

Name:
Soichiro Morishita

Affiliation:
Research Associate, RACE (Research into Artifacts, Center for Engineering), The University of Tokyo

Address:
5-1-5 Kashiwanoha, Kashiwa, Chiba 277-8568, Japan

Brief Biographical History:
2003- Received M.E. degree in information systems engineering from Saitama University
2005- Research associate, RACE, The University of Tokyo

Main Works:
• S. Morishita, H. Yokota, H. Asama, R. Himeno, and T. Mishima, "Compensation method for quantitative observation of multicolor fluorescence with nonlinear mapping," Proc. of SPIE, 7075 XI, Mathematics of Data/Image Pattern Recognition, Compression, and Encryption with Applications, pp. 70750J-1-70750J-10, 2008.

Membership in Academic Societies:
• The Institute of Electronics, Information and Communication Engineers (IEICE)
• The International Society for Optical Engineering (SPIE)

Name:
Kazuhiro Hotta

Affiliation:
Assistant Professor, Department of Information and Communication Engineering, The University of Electro-Communications

Address:
1-5-1 Chofugaoka, Chofu-shi, Tokyo 182-8585, Japan

Brief Biographical History:
1999- Research Fellow of the Japan Society for the Promotion of Science
2002- Research Associate of The University of Electro-Communications
2007- Assistant Professor of The University of Electro-Communications

Main Works:
• K. Hotta, "Adaptive Weighting of Local Classifiers by Particle Filter for Robust Tracking," Pattern Recognition, Vol.32, No.5, pp. 619-628, Elsevier, 2009.
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Membership in Academic Societies:
• The Institute of Electrical and Electronics Engineers, Inc. (IEEE)
• The Institute of Electronics, Information and Communication Engineers (IEICE)
• The Information Processing Society of Japan (IPSJ)
• Japanese Academy of Facial Studies (JFACE)

Name:
Hiroshi Takemura

Affiliation:
Assistant Professor, Department of Mechanical Engineering, Tokyo University of Science

Address:
2641 Yamazaki, Noda, Chiba 278-8510, Japan

Brief Biographical History:
2004- Guest Lecturer of Industrial Applications of Computer Science and Micro Systems, Universitat Karlsruhe
2005- Assistant Professor of the Department of Mechanical Engineering, Tokyo University of Science
2007- Visiting Researcher of RIKEN (The Institute of Physical and Chemical Research)
2008- Visiting Scholar of the Department of Mechanical Engineering, University of Michigan

Main Works:
• "Slip-adaptive Walk of Quadruped Robot," Journal of Robotics and Autonomous Systems, Vol.53, No.2, pp. 124–141, 2005.

Membership in Academic Societies:
• Institute of Electrical and Electronics Engineers, Inc. (IEEE)
• The Japan Society of Mechanical Engineers (JSME)
• The Robotics Society of Japan (RSJ)

Name:
Taketoshi Mishima

Affiliation:
Professor, Graduate School of Science and Engineering, Saitama University

Address:
255 Shimo-Okubo, Sakura-ku, Saitama-shi, Saitama 338-8570, Japan

Brief Biographical History:
1974- Research Scientist of ETL (Electrotechnical Laboratory, AIST, MITI)
1979- Senior Research Scientist of ETL (Electrotechnical Laboratory, AIST, MITI)
1995- Professor of Information and Computer Science at Saitama University

Main Works:
• "Evaluation of protein crystallization states based on texture information derived from greyscale images," Acta Crystallographica Section D, Volume 61, Part 7, pp. 873-880, Jun. 2005.

Membership in Academic Societies:
• Institute of Electrical and Electronics Engineers, Inc. (IEEE)
• Information Processing Society of Japan (IPSJ)
• Mathematical Society of Japan (MSJ)

Name:
Hajime Asama

Affiliation:
Professor, RACE (Research into Artifacts, Center for Engineering), The University of Tokyo

Address:
Kashiwanoha 5-1-5, Kashiwa-shi, Chiba 277-8568, Japan

Brief Biographical History:
1986- Research Associate of RIKEN (The Institute of Physical and Chemical Research)
1998- Senior Scientist of RIKEN (The Institute of Physical and Chemical Research)
2002- Professor of RACE (Research into Artifacts, Center for Engineering), The University of Tokyo

Main Works:
• "Distributed Task Processing by a multiple Autonomous Robot System Using an Intelligent Data Carrier System," Intelligent Automation and Soft Computing, An International Journal, Vol.6, No.3, pp. 215-224, 2000.

Membership in Academic Societies:
• Institute of Electrical and Electronics Engineers, Inc.(IEEE)
• The Japan Society of Mechanical Engineers (JSME)
• The Robotics Society of Japan (RSJ)
• The Japanese Society of Instrumentation and Control Engineers (SICE)

Name:
Haruhisa Takahashi

Affiliation:
Professor, Department of Information and Communication Engineering, University of Electro-Communications

Address:
1-5-1 Chofugaoka, Chofu-shi, Tokyo 182-8585, Japan

Main Works:
• H. Takahashi, E. Tomita, and T. Kawabata, "Separability of Internal Representations in Multilayer Perceptrons with Application to Learning," Neural Networks 6, pp.689-703, 1993.
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Membership in Academic Societies:
• International Neural Network Society (INNS)
• The Institute of Electronics, Information and Communication Engineers (IEICE)

Name:
Hiroshi Mizoguchi

Affiliation:
Professor, Department of Mechanical Engineering, Faculty of Science and Technology, Tokyo University of Science

Address:
2641 Yamazaki, Noda, Chiba 278-8510, Japan

Brief Biographical History:
1985- Research & Development Center, TOSHIBA Corp.
1994- Research Center for Advanced Science and Technology, The University of Tokyo
1997- Department of Information and Computer Science, Saitama University
2002- Department of Mechanical Engineering, Tokyo University of Science
2003- Visiting Researcher, DHRC, AIST
2007- Visiting Researcher, RIKEN (The Institute of Physical and Chemical Research)

Main Works:
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• The Robotics Society of Japan (RSJ)