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EXAMINATION OF AN IMAGE ANALYSIS SYSTEM FOR COLLECTION OF SCALE PATTERN DATA

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ABSTRACT

Image collection and measurement components and hardware settings (light control, diaphragm opening) of the Optical Pattern Recognition System (OPRS) were tested for effects on data collected for scale pattern analysis. Measurement errors up to 4% were found in the imaging components of the system. These errors appeared to have little effect on classification accuracies of scales from two stocks when compared to data collected from the same scales on a digitizer system used in previous studies. A set of standard parameters and equipment settings for the OPRS is suggested for INPFC studies. Continued testing of the OPRS for INPFC scale pattern studies is recommended.

EXAMINATION OF AN IMAGE ANALYSIS SYSTEM FOR COLLECTION OF SCALE PATTERN DATA

INTRODUCTION

Since 1978 a considerable portion of high seas salmonid research by the United States and Japan has been directed to determine the continental origins of several species of salmonids in the area of the Japanese landbased driftnet (LBDN) salmon fishery (south of 46°N) through scale pattern studies. Scientists of the Fisheries Agency of Japan (FAJ) have manually taken measurements from projected scale images in their studies (Ishida et al. 1984; Ito et al. 1985, 1986; Kato and Ishida 1985, 1986). Fisheries Research Institute (FRI) workers have collected scale pattern data on a custom-built digitizing system (Cook et al. 1980, 1981; Myers et al. 1981, 1984; Walker and Davis 1983; Walker and Harris 1982).

In 1986 the Annex to the Protocol of the International Convention for the High Seas Fisheries of the North Pacific Ocean was changed to establish a new regulatory regime for the high seas salmon fisheries. A Memorandum of Understanding on Research pursuant to the revised Annex calls for agreement among member nations of the International North Pacific Fisheries Commission (INPFC) on methodology for future scale pattern research. In an attempt to standardize data collection methods, both FAJ and FRI have purchased identical image analysis systems. This study is an examination of some of the features and limitations of that system, and a comparison of the system with equipment previously used by FRI. One goal of the study was to identify features that may introduce variability to measurements, so that equipment settings and measurement methodologies can be standardized between INPFC scale measurement labs. A second goal was to evaluate the stock separation accuracy of data from the OPRS in comparison with accuracies obtained from data collected on a digitizing system at FRI.

MATERIALS AND METHODS

A. Materials

The image analysis system tested was the Optical Pattern Recognition System (OPRS)[1] Model OPR-512, manufactured by BioSonics, Inc. It consists of a microscope, video camera, video frame grabber, video monitor, microcomputer, and digitizer tablet. The scale image is acquired through the microscope by the video camera. The frame grabber, located in the microcomputer, transforms the video image into a digital

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[1]Use of brand-name products does not imply endorsement by FRI or contracting agencies.

data array and displays the image on the monitor screen. Since American and European video standards employ a non-square image with a horizontal to vertical ratio of 4:3, the OPRS corrects for this by mapping the pixel array onto a higher resolution virtual coordinate system with a 1:1 ratio (BioSonics 1987). Measurements are made from the virtual coordinate system. A digitizing pad, linked to the computer, allows interaction with the image on the screen for operations such as marking locations for measurement. The location of the digitizing cursor is displayed on the monitor. In the OPRS the digitizing pad acts only as a pointing device or mouse, and not as a measuring device. Data acquisition is handled by a menu-driven proprietary program.

The specific models of components in the system acquired by FRI in September 1986 are as follows: Olympus BHTU microscope with 4X, 10X, and 20X D Plan objective lenses and 1X and 2X S Plan objectives, monocular observation tube, FK adapter, MTV-3 parfocalizing adapters, and NFK 2.5X photocompensating eyepiece; RCA TC2511 black and white video camera; Panasonic MT-1340G color video medical monitor; Matrox PIP-512 video digitizer board (frame grabber); and GTCO Micro DIGI-PAD model MD7-0606. To the microscope FRI added an Olympus magnification changer with 1X, 1.25X, and 1.5X powers and replaced the microscope slide holder with a custom-built glass holder for acetate cards. The microcomputer used is a Compaq Deskpro 286 model 2550.

No system specifications were provided with the OPRS. Specifications for the video camera include a geometric distortion of 1.5% maximum of picture height within a center circle with a diameter equal to the picture height and 2% overall. Specifications for other components did not include similarly explicit measures of distortion or error. Measurements and errors reported in this study may apply only to the OPRS at FRI and may not apply to systems with different component models or even to systems with the same models, due to variation between individual units.

In the FRI digitizer system, a custom-built cabinet holds a translucent Talos digitizing tablet (model RP 614 B); a projection lamp shines light through scale impressions and a Micron 15 mm lens onto mirrors in the cabinet which project the image onto the rear surface of the digitizing tablet. Resulting magnification is 104X. Data coordinates from the tablet are passed via custom-designed buffers and computer circuit boards to a Vector 1++ microcomputer. Data acquisition and storage are handled by DIGSCALE, a program written for FRI in the computer language C.

No system specifications were provided with the FRI digitizer. The digitizing tablet has a resolution of 1000 lines per inch with an accuracy of $\pm$ 0.005 inch.

Two micrometers were used in the tests: an Olympus OB-M-1/100 and a Reichert 1400. The FRI scale archives provided a coho salmon scale used in some tests of equipment settings (Nushagak River, 8/7/80, card 001, fish number 09).

In addition to data collection software, several other programs were used in this analysis. Data from both systems were reformatted with FORTRAN programs and were analyzed with BMDP statistical routines (Dixon et al. 1983).

B. Methods

1. OPRS Microscope, Camera, and Image Mapping Components

Precision of measurements collected through these components was tested through repeated measurement of a 1 mm segment of the Reichert micrometer at 2X, 4X, and 6X objective powers, at various orientations of the micrometer image on the monitor, and at various locations of the image on the monitor screen. The micrometer image was oriented horizontally, vertically, and diagonally by rotation of the micrometer on the microscope stage and/or by rotation of the video camera. Measurements were made with the image in the center of the screen, in all four corners, and at the edges of the screen.

2. FRI Digitizer Projection System

Precision of measurements collected through the digitizer lens and projection mirrors was tested through repeated measurement of a 1 mm segment of the Reichert micrometer with a 104X lens, with the micrometer image oriented horizontally and vertically on the digitizing tablet, and at the center, corners, and edges of the tablet. The micrometer image was oriented by rotation of the micrometer in the sample holder and by rotation of the mirrors in the cabinet holding the digitizing tablet.

3. Digitizing Tablets

Precision of measurements collected from the digitizing tablet in the FRI digitizing system was tested through repeated measurements across sets of ten detection wires (total distance of two inches) embedded in the digitizing tablet. Sets of wires were measured both horizontally and vertically in the center and in the corners of the tablet. Precision of measurements collected from the OPRS digitizing pad is not important, as the digitizing pad is used only as a pointing device.

4. OPRS Hardware Settings

Effects of different light levels, controlled by hardware settings, were tested by repeated measures of the same salmon scale at various settings. The voltage control was set at three levels, corresponding to average, slightly darker-than-average, and substantially darker-than-average images on the monitor, in a narrow range at less than two volts. The light control lever was moved within a 5 mm range, approximately 17 mm back from the forward, or off, position. This produced no change on the indicator panel, or voltmeter, on the front of the microscope, which remained at the lowest position. Settings outside this range yielded light levels that could not be

accommodated by the video camera. The automatic gain control feature of the camera corrected brighter light levels back to a standard level; very bright light completely washed out the image. Completely dark images were obtained at a position a short distance below the setting for a normal image. Aperture and field iris diaphragms were set at dark (aperture setting .9, field setting 0), average (midpoint for both diaphragms), and bright (aperture setting .1, field setting 9). Counts of circuli were compared for scales measured at different light levels to determine if the difference had caused the circulus detection feature of the software to miss or add circuli.

A glass holder for acetate cards was constructed for FRI to replace the microscope slide holder. An identical holder has been provided to FAJ. Measurements of a test scale were made with and without the glass holder, and circulus counts and intercircular distances were examined for differences.

5. OPRS Software Settings

Smoothing parameters, weighting factors for running averages used for light/dark discrimination, were set at 1 (for the -1, or immediately preceding, sampling unit), 3 (for the center sampling unit), -1 (for the +1, or next, sampling unit), and -3 (for the +2 sampling unit). Smoothing parameters for all other sampling units were set to zero. The small number of parameters used (out of 13 possible) is designed to work over short intercircular distances at 4X and 6X objective lens combinations. The asymmetrical pattern of the parameters shifts the automatic circulus mark to the outer edge of the circulus. Hysteresis (level of detection of difference between bright and dark regions) was set at 15 luminance units (in a range of 256). The 'Minima' item was selected to cause the circulus marks to be placed on dark bands (circuli) rather than on the brighter intercircular spaces. Gain (brightness) and offset (contrast) factors were set at 110 and 30, respectively. Lens calibration factors (in meters per virtual unit) were $6.25390869 \times 10^{-8}$ for the 6X lens combination and $9.26784059 \times 10^{-8}$ for the 4X combination. No other lenses were used for measurements. The standard OPRS version 1.08 conversion factor of 51.6007708 virtual units per sampling unit was used. Color control (output Look Up Tables, or LUT) was modified to create a light purple background color on the screen; a green overlay was used for lines and axes. All OPRS settings are summarized in Table 1.

No testing of software settings was done. Several of these settings will have a significant impact on scale measurements. For further consideration of these settings, see the Discussion section below.

6. Scale Measurement Procedure

On the OPRS, scale images were oriented diagonally on the monitor screen by rotation of the video camera; the focus of the scale was placed toward the lower left corner. A reference line for the

measurement axis was drawn at 4X objective power across the posterior ends of the first marine annulus. Measurement of freshwater growth from the focus through the freshwater annulus was made at 6X; measurement of growth from the freshwater annulus to the end of the first marine annulus was made at 4X. For both measurements, the image of the freshwater zone was enhanced with the Sharpening 1 feature of the OPRS. Measurements were made along an axis oriented 90° to the reference line.

Measurement of scales on the digitizer system was similar to technique used in previous studies. Scales were projected at 104X and oriented with the posterior tips of the first marine annulus touching a pre-drawn reference line. Measurements were made from the focus through the first marine annulus along an axis 90° to the reference line.

One person, the author, made all measurements discussed in this study.

7. Scale Measurements and Stock Separation

Chinook salmon scales used in previous continent of origin studies (Myers et al. 1984) were remeasured on both the OPRS and FRI systems. One hundred scales were measured from both the Asia (ASIA) and western Alaska (WEST) standards, from 118 (ASIA) and 198 (WEST) scales previously used. Care was taken to align the measurement axis of the scale the same way on the two systems. An exact duplication of measurements was not attempted; rather, scales were measured on each system in the manner a worker would normally use on that equipment. This depended primarily on the clarity of the image on each system. For easier comparison, data from the OPRS were converted from sampling units (roughly equivalent to picture elements, or pixels, on a video screen) to thousandths of an inch, the units used by the digitizer system. Data from both systems were reformatted to measurements used in previous scale pattern analyses. Paired sample t-tests (program BMDP 3D) were run on 13 reformatted measurements to detect significant differences between measurements made on the two machines.

The data analysis portion of the OPRS software was not evaluated for this study, partially because of the difficulty in using it. No documentation of these features was provided. At present only one method of stock separation (linear discriminant function, or LDF) is fully functional, of three methods (linear, quadratic, and polynomial discriminant functions) listed on the menu for the pattern recognition module. The LDF program provided lacks the flexibility of LDF programs in statistical packages such as BMDP and SPSS. A built-in set of scale characters is used, so that creation and selection of variables is difficult.

Four different linear discriminant analyses (BMDP 7M), each using a different variable set (Table 2), were run on each data set. The variable sets used were (1) the complete set of 48 scale characters used in previous studies (Myers et al. 1984), (2) a reduced set of 11 characters which eliminates highly correlated and non-normally

distributed characters (Davis 1987), (3) a set of five freshwater and twelve marine triplets (triplets are measurements across three adjacent circuli), and (4) a set using only those triplets from the reduced set of 11 (one freshwater and six marine). Variable sets (3) and (4) were chosen because small distance measurements, such as triplets, were believed to be more sensitive to measurement error than characters such as size or circulus count of entire zones of the scale. The same four analyses were also run on data collected from the same scales by six different readers on the digitizer system in a previous study (Myers et al. 1984). Only classification of the standard ASIA and WEST samples was evaluated. No mixed samples or unknowns were classified.

Scale characters were examined to determine which characters used in this stock separation might be sensitive to small measurement errors. The percentage difference between means for the two stocks used was calculated for those characters selected for the linear discriminant functions of the four analyses, and the magnitude of the difference was compared to the size of the measurement errors. Percentage difference was calculated as difference between mean values for western Alaska and Asia, divided by the value for Asia, times 100%.

The amount of time required to measure a certain number of scales was noted, after the reader had achieved comparable levels of proficiency on both systems.

RESULTS

A. Measurement Errors

Exploratory measurements of micrometer segments showed that there were differences between measurements taken at the center of the OPRS monitor screen and those taken in the corners and at the edges, and that the differences became greater farther from the center. Additional measurements taken at one corner of the screen demonstrated that the distortion occurs with different lenses, with different powers of the magnification changer, and for both horizontal and vertical images (Table 3). Horizontal images in the center of the screen became slightly diagonal when moved to the corners and had to be corrected to horizontal by rotation of the camera. For horizontal images, the magnitude of the difference between means of center and corner measurements of a 1 mm micrometer segment was 4.2% for 2X, 3.7% for 3X, and 2.3% for 4X; at 3X the difference for vertical images was 1.8%. The differences were greater for lower magnifications than for higher ones, and greater for horizontal images than vertical ones. This is probably because the image is more distorted farther from the center; a lower power lens projects a smaller image and thus a greater proportion of that image can be placed in the corner, farther from the center. Similarly, because of the horizontal, rectangular shape of the screen, a larger portion of a horizontal image can be distant from the center than of a vertical image.

Because the distortion occurs with different objective lenses and with different powers of the magnification changer, it seems likely that much of the distortion is due to the optics of the video camera, or possibly the lenses in the microscope adapter for the camera. The objective lenses and magnification changer may also contribute to the distortion.

There was also 2% difference between horizontal and vertical measurements of the same 1 mm micrometer segment. Regardless of how the orientation of the image on the monitor screen was achieved, vertical measurements were larger. Rotating the camera so that the horizontally-placed micrometer appeared vertically on the screen yielded a 1.9% larger measurement. Leaving the camera in horizontal attitude and placing the micrometer vertically beneath the objective gave a 2.0% larger measurement. If the camera were then rotated so that the image was shifted back to horizontal, the length was close (0.5% smaller) to that measured horizontally.

Measurement errors associated with the older digitizer system are much smaller. Differences between 1 mm micrometer images placed at the center and corners of the digitizing tablet range from 0.1% to 0.8%, and differences between vertical and horizontal images are on the order of 0.2% and 0.6% (Table 4). Measurements testing the mirror projection and rotation system yielded differences in image size ranging from 0.1% to 0.3% (Table 5). Variations in measurements taken directly off features of the digitizing tablet were also small; distances across sets of ten detection wires, both horizontally and vertically and in the center and corners of the tablet, differed by 0.0% to 0.3% (Table 6).

B. OPRS Hardware Settings

Within a reasonable range, different light settings had very little effect on the circulus detection features of the OPRS. The automatic gain control of the video camera adjusts small changes in light to a standard level, and a bright or washed out image is almost impossible to achieve without saturating the camera and completely obliterating the image. When pushed to levels that are obviously darker than normal, the OPRS begins to lose detection of small or closely spaced circuli. At darker light settings, two or three circuli out of 50 were missed on a test scale image (Table 7).

Settings of the diaphragms that control light passing to the objective lens also had little effect on circulus detection, except at very dark settings. When either diaphragm was wide open, the number of circuli detected was the same as when the diaphragms were in the middle position (Table 7). When diaphragms were stopped down to the extreme dark position, approximately seven circuli out of 53 were not detected on the test scale image and variability in number of circuli detected increased (Table 7).

The glass holder for acetate cards had no influence on scale measurements. Intercircular distances and circuli counts were

essentially identical for measurements made with and without the acetate card holder.

C. Scale Pattern Analyses

Although no attempt was made to make scale data collected on the OPRS and digitizer systems identical, the data files were nevertheless very similar. Paired sample t-tests showed three variables out of 13 to be different ($p<0.05$) between the two ASIA files; no variables were found to be significantly different between the two WEST files. The three variables found to be different between the files for ASIA were size of freshwater zone, size of first ocean zone, and size of first triplet in the ocean zone. The mean values of the differences for these characters were 0.018, 0.066, and 0.015 inches at 104X, respectively, which correspond to 1.38, 3.37, and 0.77 sampling units at the magnifications at which the data were collected on the OPRS. A sampling unit is roughly the size of a pixel, or video picture element.

Classification accuracies, for analyses for each of the four variable sets, were also very similar for data from the two systems (Table 8). Overall accuracies for the digitizer data were always slightly higher, but only by 0.5% to 3.0%. For both of the data sets, many of the same scale characters were selected by the discriminant program, particularly for the first few variables. Overall accuracy generally improved very little after entry of the first character in the discriminant model. The primary stock discriminators in both cases were size of ocean zone and scale size through the first marine annulus, and sizes of the first freshwater triplet and of the first two marine triplets. When the same four analyses were run on data from the same scales collected for a previous analysis (Myers et al. 1984), similar accuracies were achieved and the same characters were the primary discriminators.

Examination of the percent difference between the two stocks for the characters used in the discriminant analyses revealed that stocks generally differed by 15% or more (Table 9). However, two characters (C39, first freshwater triplet, and C52, fourth marine triplet) which were selected in several of the analyses differed by only 3-5%. This difference is within the magnitude of some measurement errors on the OPRS.

Measurement of scales on the OPRS took approximately five minutes per scale, roughly twice as long as on the digitizer system. This was primarily due to the necessity of measuring the freshwater and marine portions of the scale separately, at two different magnifications. Video image quality did not permit sufficient resolution of freshwater circuli at a magnification that also allowed display of the entire first marine year on the screen. Other time-consuming aspects of the OPRS were setting a reference line at a different magnification than that used for the following freshwater measurement, and checking the automatic circulus designations to ensure that no marks were left out or added.

DISCUSSION

Scale data collection on the OPRS did not seem to substantially affect stock separation analysis on the single sample tested (Table 8). However, the magnitude and nature of the measurement errors detected in the OPRS acquired by FRI (Table 3) were not anticipated. Though the differences are relatively small (on the order of 2 to 5%), they could potentially affect scale data if applied in a differential or systematic way, and could especially have an effect on data exchanged between two laboratories if the data were collected in different manners.

Differences between horizontal and vertical measurement can be controlled by standardizing orientation of the scale image on the screen. For data collected on different OPRS units to be compared, users of the different systems must agree on and use the same orientation. At FRI we plan to use a diagonal measurement axis from lower left to upper right.

The differences between measurements in the center and those toward the edges and corners of the screen and of the digitizing pad are more difficult to compensate for. An obvious remedy is substitution of a higher quality video camera and microscope optics with less distortion. To use the system with the components provided to FRI, workers should place scale images in the same area of the screen. However, if all scales being measured are not of the same size, larger scales will extend beyond the standard area. For example, a smaller scale may extend from the corner of the screen to the center of the screen, while a larger scale may extend entirely across the screen toward the opposite corner. Measurements at the edge of the smaller scale will be made at the center of the screen, and thus be relatively larger than measurements of the edge of the larger scale, which will be made toward a corner. In all cases, workers should avoid taking measurements on the the periphery of the screen.

A further problem arises if data are to be compared to or used with data collected outside of the OPRS (e.g., back-calculated body lengths). Lengths calculated from data collected at the center of the screen will be relatively longer than lengths calculated from data collected toward the edges or corners of the screen.

Another circumstance to be aware of is the magnitude of some of the errors in comparison to the differences between stocks of some of the scale characters used for stock separation. While most characters used for separation showed differences of 15% or more between stocks, a few differed by only 3% or 4%, a difference similar in size to the center-edge distortion. If this distortion applied differentially to one of the stocks, as might happen in the example of stocks of dissimilar scale size mentioned above, characters with small between-stock differences could be affected.

The types and magnitudes of errors described above may only apply to the components in the OPRS system acquired by FRI. However, other

users should be aware of the potential for such errors and should make test measurements and calibrations appropriate to their application. In addition, I would strongly urge BioSonics to undertake a thorough evaluation of the sources and magnitudes of the various distortions and measurement errors possible on the OPRS and to provide these specifications to users. Knowledge of types and magnitudes of errors would allow users to plan data collection methodologies and routines to avoid effects of some of the errors.

The hardware settings tested (light level, diaphragm openings) have little effect on measurements until they begin to change the image noticeably. The glass used in the acetate card holder also appears to have no effect on measurements. Some software parameters not tested here are similarly believed to have little or no effect on measurements when set at levels that give reasonable images. Among these are the gain and offset settings and the background color set on the output look up tables.

However, several of the parameters in the OPRS software can substantially change the data. They were not tested for this study, in part because their effect is almost self-evident. Calibration of the lenses is very important. It is difficult to achieve a repeatable calibration of a lens. Part of the problem is due to the inherently fuzzy nature of the video image: it becomes difficult to place the start and end of the calibration line at the same place each time. Another difficulty lies in the interaction of the screen, digitizing pad, and virtual coordinate system. The digitizing pad, which has a higher resolution than the screen, can mark starting or ending locations of the micrometer at slightly different locations in the virtual coordinate system, though the locations may be displayed at the same pixel coordinate on the screen. Thus, very small changes in location on the pad may not be reflected on the screen, but can affect the length of the calibration line. Workers exchanging data between OPRS units should exchange data in raw, sampling unit format and provide calibration factors for the lenses used.

The smoothing parameters for light-dark discrimination have a major effect on the number and location of circuli detected. The ones used for this study are designed to be effective over short distances and to shift the circulus tick mark to the outer edge of the circulus. The hysteresis level, which sets the sensitivity to light-dark differences, is also important. Both of these factors should be standardized between laboratories. Sharpening the image, and the type of sharpening, can affect the data collected from a scale image, though not to as great an extent as the parameters discussed above.

Although the OPRS is essentially a turnkey system, it is obvious that some of the software settings will require modification by the user. The user should explore different values for smoothing parameters and hysteresis level to see what settings work best for a particular application. The summary list of settings used in this study (Table 1)

is offered as a starting point for a standardized set of parameters for INPFC scale pattern researchers working with the OPRS.

The menu-driven software provides the flexibility needed for many kinds of data collection and offers a wide variety of useful features, but some first-time users of the system have had difficulty learning the appropriate pages and commands and recalling all of the steps needed to measure scales. Despite the ease of use of the menu format, the complexity and array of options and parameters on the OPRS may require a longer training and learning period than simpler methods of scale measurement.

A final factor is the question of reader variability. The scale reader has a major influence on the data collected on both the FRI digitizer and the OPRS, in spite of the many automated features of the systems. Setting the reference line and measurement axis, determining the starting point for measurements, and determining the boundaries of scale zones are all subjective decisions made by the reader. Through a rigorous program of consultation and group consensus, these decisions can reach a fairly high degree of standardization. Such consensus requires continuing effort, and is particularly difficult to obtain between laboratories. Until there is a reliably high level of standardization, measurement of scales for analysis of each age class or brood year should be carried out by a single reader, or by a small group of readers working closely together.

Performance of OPRS data in the stock separation comparison was comparable to that of data from the FRI digitizing system, though one must be cautious in generalizing from a single test of two stocks. In most instances, the same variables were selected for discrimination between the stocks, even when the data set was limited to scale characters representing short distance measurements. Short distance measurements might be expected to show more variation between systems than measurements of entire life history zones. Classification accuracies for the OPRS data were only slightly lower than for the digitizer data. Of more concern was the longer time required to collect data on the OPRS, due to the necessity of measuring two portions of the scale at separate magnifications.

For collection of routine scale pattern data, the OPRS seems to perform adequately. Measurement errors did not appear to seriously affect stock separation analysis. At FRI we have reservations about the much slower rate at which scales can be measured in comparison to an older digitizer system, but we plan to use the OPRS for stock separation studies during the next year. I recommend continued testing of the OPRS for INPFC scale pattern studies.

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Table 1. OPRS settings and parameters used in this study. Asterisks (*) denote features with most effect on measurements.

	Feature	Setting
	Software:	
*	Smoothing parameters	(-2): 0 (-1): 1 (center): 3 (+1): -1 (+2): -3 (+3): 0
*	Hysteresis	15
	Amplification	Manual
	Gain	110
	Offset	30
*	Calibrations (meters per virtual unit): 4x (4X objective, 1.0X mag. changer) 6X (4X objective, 1.5X mag. changer)	$9.26784059 \times 10^{-8}$ m/v.u. $6.25390869 \times 10^{-8}$ m/v.u.
	Virtual units per sampling unit conversion (built-in to OPRS version 1.08)	51.6007708 v.u./s.u.
	Maxima/Minima (choice of light or dark bands to place circulus tick marks on)	Minima
*	Sharpening feature used on freshwater growth	Sharpening 1
	Overlay	Green
	Background color (Look Up Table [LUT] Sub-Page)	Light purple
	Red Output LUT	normal ramp to 90% of max
	Green Output LUT	zero
	Blue Output LUT	normal ramp to max.
	Microscope hardware:	
	Light (voltage control lever)	< 2 volts (approximately 17 mm from forward end)
	Field (lower) diaphragm	midway ("5")
	Aperture (upper) diaphragm	midway (0.5)

Table 2. Forty-eight scale characters calculated for use in the scale pattern analyses. R: reduced variable set; T: triplets.

	Character No.	Description[a]
R	C1	Size Zone 1
R	C5	Size Zone 2
	C6	Size Zones 1 + 2
	C7	No. circuli Zones 1 + 2
	C9	(Size Zones 1 + 2)/(no. circuli Zones 1 + 2)
	C11	Size Zone 2/(size Zones 1 + 2)
R	C12	No. circuli Zone 1
R	C16	No. circuli Zone 2
	C17	Size Zone 1/no. circuli Zone 1
	C21	Size Zone 2/no. circuli Zone 2
	C22	Distance circuli 1 to 3 in Zone 2/(size Zones 1 + 2)
	C23	Distance circuli 4 to 6 in Zone 2/(size Zones 1 + 2)
	C24	Distance circuli 7 to 9 in Zone 2/(size Zones 1 + 2)
	C25	Distance circuli 10 to 12 in Zone 2/(size Zones 1 + 2)
	C26	Distance circuli 13 to 15 in Zone 2/(size Zones 1 + 2)
	C27	Distance circuli 16 to 18 in Zone 2/(size Zones 1 + 2)
	C28	Distance circuli 19 to 21 in Zone 2/(size Zones 1 + 2)
	C29	Distance circuli 22 to 24 in Zone 2/(size Zones 1 + 2)
	C30	Distance circuli 25 to 27 in Zone 2/(size Zones 1 + 2)
	C31	Distance circuli 28 to 30 in Zone 2/(size Zones 1 + 2)
	C32	Distance circuli 31 to 33 in Zone 2/(size Zones 1 + 2)
	C33	Distance circuli 34 to 36 in Zone 2/(size Zones 1 + 2)
	C34	Distance circuli 1 to 9 in Zone 2 (= character Nos. C49+C50+C51)
	C35	Distance circuli 10 to 18 in Zone 2 (= character Nos. C52+C53+C54)
	C36	Distance circuli 19 to 27 in Zone 2 (= character Nos. C55+C56+C57)
	C37	Distance circuli 28 to 36 in Zone 2 (= character Nos. C58+C59+C60)
RT	C39	Distance circuli 2 to 4 in Zone 1
T	C40	Distance circuli 5 to 7 in Zone 1
T	C41	Distance circuli 8 to 10 in Zone 1
T	C42	Distance circuli 11 to 13 in Zone 1
T	C43	Distance circuli 14 to 16 in Zone 1
	C44	Distance circuli 2 to 4 in Zone 1/(size Zones 1 + 2)
	C45	Distance circuli 5 to 7 in Zone 1/(size Zones 1 + 2)
	C46	Distance circuli 8 to 10 in Zone 1/(size Zones 1 + 2)
	C47	Distance circuli 11 to 13 in Zone 1/(size Zones 1 + 2)
	C48	Distance circuli 14 to 16 in Zone 1/(size Zones 1 + 2)

Table 2. Forty-eight scale characters calculated for use in the scale pattern analyses. R: reduced variable set; T: triplets - continued.

Character No.		Description[a]
RT	C49	Distance circuli 1 to 3 in Zone 2
RT	C50	Distance circuli 4 to 6 in Zone 2
RT	C51	Distance circuli 7 to 9 in Zone 2
RT	C52	Distance circuli 10 to 12 in Zone 2
RT	C53	Distance circuli 13 to 15 in Zone 2
RT	C54	Distance circuli 16 to 18 in Zone 2
T	C55	Distance circuli 19 to 21 in Zone 2
T	C56	Distance circuli 22 to 24 in Zone 2
T	C57	Distance circuli 25 to 27 in Zone 2
T	C58	Distance circuli 28 to 30 in Zone 2
T	C59	Distance circuli 31 to 33 in Zone 2
T	C60	Distance circuli 34 to 36 in Zone 2

[a]Zone 1: The area of the scale from the center of the focus to the outer edge of the last circulus in the freshwater annulus; first year of growth;
Zone 2: The area of the scale from the outer edge of the last circulus in the freshwater annulus to the outer edge of the last circulus in the first ocean annulus; second year of growth.

Table 3. Measurements of 1 mm micrometer segments on OPRS monitor screen. Measurements are in sampling units. H = horizontal; V = vertical; CT = center; SE = southeast corner;
A = percent difference of mean corner measurement from center measurement at same power and orientation;
B = percent difference of mean measurement from horizontal measurement at center, 3X.

Orientation			Screen	Lens						
image	microm.	camera	area	power	N	$\bar{x}$	s.d.	Range	A	B
H	H	H	CT	3X	25	152.88	0.33	152-153		
H	H	H	SE	3X	25	147.20	0.58	146-149	3.7%	
H	H	H	CT	2X	25	101.64	0.49	101-102		
H	H	H	SE	2X	25	97.40	0.65	96-99	4.2%	
H	H	H	CT	4X	25	207.04	0.45	206-208		
H	H	H	SE	4X	25	202.20	0.65	201-203	2.3%	
V	H	V	CT	3X	25	155.80	0.50	155-157		1.9%
V	H	V	SE	3X	25	152.92	0.64	152-154	1.8%	
V	V	H	CT	3X	25	155.96	0.35	155-157		2.0%
H	V	V	CT	3X	25	152.12	0.78	151-153		-0.5%

Table 4. Measurements of 1 mm micrometer segments on FRI digitizer: test of projection. Measurements are in thousandths of an inch. H = horizontal; V = vertical; CT = center; SE,SW = compass designations for corners of the tablet;
A = percent difference of mean corner measurement from center measurement at same orientation;
B = percent difference of mean measurement from horizontal measurement at same area of tablet.

Orientation image	Orientation microm.	Orientation mirror	Tablet area	N	$\bar{x}$	s.d.	Range	A	B
H	H	H	CT	25	4135.72	4.81	4128-48		
H	H	H	SE	25	4140.44	3.15	4135-47	0.1%	
H	H	H	SW	25	4153.28	2.91	4148-59	0.4%	
V	H	V	CT	25	4110.92	2.31	4106-16		0.6%
V	H	V	SW	25	4143.12	2.33	4139-49	0.8%	0.2%

Table 5. Measurements of 1 mm micrometer segments on FRI digitizer: test of orientation mirrors. Micrometer was shifted in 45° steps through 180°. Measurements are in thousandths of an inch. V = vertical; A = percent difference of mean measurement from starting vertical measurement.

Orientation image	Orientation micrometer	N	x	$\bar{s}$.d.	Range	A
V	0°	25	4104.24	2.31	4100-109	
V	45°	25	4099.60	4.65	4093-109	-0.1%
V	90°	25	4107.32	5.59	4099-116	0.1%
V	135°	25	4105.20	4.37	4105-120	0.2%
V	180°	25	4115.92	4.20	4108-123	0.3%

Table 6. Measurements of sets of ten embedded wires (2 inches) on FRI digitizer: test of digitizing tablet. Measurements are in thousandths of an inch. H = horizontal; V = vertical; CT = center; SE,SW,NW,NE = compass designations for corners of the tablet; A = percent difference of mean measurement from center measurement at horizontal orientation.

Orientation	Tablet area	N	$\bar{x}$	s.d.	Range	A
H	CT	5	2004.2	4.3	1999-2008	
H	SE	5	1998.6	3.2	1996-2004	-0.3%
H	SW	5	2005.0	2.9	2003-2010	0.0%
H	NW	5	2005.0	1.9	2002-2007	0.0%
H	NE	5	2003.0	5.3	1997-2011	-0.1%
V	CT	5	2003.2	3.3	2001-2009	-0.1%
V	SE	5	2004.4	3.8	1999-2006	0.0%
V	SW	5	2000.0	2.7	1997-2002	-0.2%
V	NW	5	2001.8	1.6	1999-2003	-0.1%
V	NE	5	2004.8	2.7	2003-2009	0.0%

Table 7. Tests of hardware settings controlling light on the OPRS. Measurements are number of circuli detected on a coho salmon scale image.

Control	Setting	N	$\bar{x}$	s.d.	Range
Light	normal	5	50.0	0.0	50
	slightly dark	5	47.0	0.0	47
	dark	5	48.0	1.4	46-49
Field diaphragm	open	5	53.0	0.0	53
	mid	5	53.0	0.0	53
	closed	5	45.4	0.9	45-47
Aperture diaphragm	open (.9)	5	53.2	0.4	53-54
	mid (.5)	5	53.0	0.0	53
	closed (.1)	5	46.2	1.8	44-48

Table 8. Classification steps and accuracies from stock separation of brood year 1973 chinook salmon from western Alaska (W) and Asia (A) scale pattern data collected on OPRS and FRI digitizer systems, with classification of data from same scales used in a previous analysis (Myers et al. 1984). Classification performed by BMDP program 7M for four different variable sets (see text for description of variable sets). Sample size for each stock is 100. Minus sign (-) indicates variable was removed at that step.

	OPRS				DIGITIZER				PREVIOUS			
Step	Char	Overall	W	A	Char	Overall	W	A	Char	Overall	W	A
Full Set:												
1	C6	80.5	87	74	C6	81.0	85	77	C6	81.0	84	78
2	C50	81.0	85	77	C50	83.5	85	82	C50	81.5	85	78
3	C39	83.5	87	80	C39	82.0	84	80	C53	82.0	85	79
4	C57	82.5	86	79	C22	82.0	83	81	C39	83.0	87	79
5	C11	80.0	84	76	C35	83.0	83	83	C37	83.5	89	78
6	C34	83.0	85	81	C59	84.5	86	83				
7	-C50	82.5	85	80								
8	C52	83.0	84	82								
Reduced Set:												
1	C5	78.5	82	75	C5	80.0	83	77	C5	77.5	79	76
2	C50	79.5	83	76	C50	83.5	86	81	C50	81.0	85	77
3	C39	83.0	84	82	C39	84.0	85	83	C53	83.0	85	81
4	C1	82.0	85	80	C49	83.5	86	81	C12	83.0	85	81
5	C52	81.0	82	80								
Triplets Only:												
1	C50	71.0	69	73	C50	74.0	71	77	C50	74.5	75	74
2	C57	82.0	83	81	C57	81.0	83	79	C57	79.0	81	77
3	C41	82.0	83	81	C49	83.0	85	81	C56	82.0	86	78
4	C39	82.0	82	82	C58	82.5	87	78	C49	81.5	84	79
5	C49	82.5	83	82	C41	82.0	86	78	C53	81.0	82	80
6	C51	82.5	84	81	C39	82.0	85	79	C54	81.5	84	79
7									C42	82.5	85	80
Triplets from Reduced Set:												
1	C50	71.0	69	73	C50	74.0	71	77	C50	74.5	75	74
2	C54	71.5	69	74	C49	74.0	73	75	C54	74.0	72	76
3	C52	74.0	73	75	C52	76.0	75	77	C53	76.0	76	76
4					C54	77.0	76	78	C49	75.0	75	75

Table 9. Percentage difference between mean values of Asian and western Alaskan standards for variables selected in stock separation analyses. Marks (x) indicate analyses in which the variables were used. F: full variable set; R: reduced variable set; T: triplets only; RT: triplets in reduced variable set. Percent difference was calculated as difference between mean values for western Alaska and Asia, divided by mean value for Asia, times 100%.

Variable	Analysis F	R	T	RT	OPRS	Analysis F	R	T	RT	Digitizer
C1		X			24.2					
C5		X			22.2		X			23.6
C6	X				22.6	X				23.3
C11	X				0.1					
C22						X				4.5
C34	X				19.1					
C35						X				8.1
C39	X	X	X		4.0	X	X	X		4.9
C41			X		150.5			X		145.2
C49			X		15.6		X	X	X	19.5
C50	X	X	X	X	26.1	X	X	X	X	26.4
C51			X		16.0					
C52	X	X		X	3.9				X	3.6
C54				X	12.7				X	12.5
C57	X		X		68.8			X		76.1
C58								X		146.4
C59						X				89.6