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Contents

Message from UTAR President 1

Message from FES Dean 2

Message from the Chair 3

Keynote Lectures and Invited Talks 4

Biodata of Invited Speakers 4

Keynote Lecture : Wavelets, Multiwavelets and Wavelet Frames for Periodic Functions
Say Song Goh 6

Keynote Lecture : Data Depth for New Nonparametric Inference Schemes and Beyond (Abstract Only)
Regina Y. Liu 22

Keynote Lecture : Stochastic Mixed Integer Nonlinear Programming (Abstract Only)
Herman Mawengkang 23

Keynote Lecture: Contemporary Statistical Data Visualization
Junji Nakano 24

Keynote Lecture: Insurance Risk Models: With and Without Dividends (Abstract Only)
Hailiang Yang 47

Invited Talk: Some One-sided Multivariate Tests
Samruam Chongcharoen 48

Invited Talk: On Dynamical Systems and Phase Transitions for $Q+1$-state p-adic Potts Model on the Cayley Tree
Farrukh Mukhamedov 68

Invited Talk: Quartic-Normal Distributions (Abstract Only)
Ah Hin Pooi 82

Invited Talk: Research Collaboration Network Analysis of the Journal of Finance (Abstract Only)
Kurunathan Ratnavelu 83

Invited Talk: The Ultimate Solution Approach to Intractable Problems
Abdellah Salhi 84

Contributed Talks : Pure Mathematics / Combinatorics / Algebra / Analysis / Graph Theory 94

Minimal Realization of BL-General Fuzzy Automata
Khadijeh Abolpour and Mohammad Mehdi Zahedi 94

Comparative Study of Geometric Product and Mixed Product
Md. Shah Alam and Sabar Bauk 110

On Chromatic Uniqueness and Equivalence of K_4-Homeomorphic Graphs
Sabina Catada-Ghimire and Roslan Hasni 115

Constructions of Non-commutative Generalized Latin Squares of Order 5
H. V. Chen, A. Y. M. Chin, and Shereen Sharmini 120

Spectral Corrections for a Class of Eigenvalue Problems
Mohamed K. El Daou 131

On Special Solvents of Some Nonlinear Matrix Equations
Yin-huan Han and Hyun-min Kim 144

n-fold Commutative Hyper K-ideals
Mona Pirasghari, Parvaneh Babari and Mohammad Mahdi Zahedi 150

On ξ^s-quadratic Stochastic Operators in 2-dimensional Simplex
Farrukh Mukhamedov and Afifah Hanum Mohd Jamal 159

Single Polygon Counting for m Fixed Nodes over Cayley Tree of Order Two
Chin Hee Pah and Mansoor Saburov 173

n-fold Positive Implicative Hyper K-ideals
Parvaneh Babari, Mona Pirasghari and Mohammad Mahdi Zahedi 186

On *μ-closed Fuzzy Sets, Fuzzy *μ-closed Maps, Fuzzy *μ-irresolute Maps and *μ-homeomorphism Mappings in Fuzzy Topological Spaces
Sadanand N. Patil 201

On Fuzzy $g\mu$-closed Maps, Fuzzy $g\mu$-continuous Maps and Fuzzy $g\mu$-irresolute Mappings in Fuzzy Topological Spaces
Sadanand N. Patil, A. S. Madabhavi, S. R. Sadugol and G. R. S. B. Madagi 214

On Graph-(super)magic Labelings of a Path-amalgamation of Isomorphic Graphs
A.N.M. Salman and T.K. Maryati 228

A Survey on Equations in Group Ring
Tai Wei Hang and Denis Wong Chee Keong 234

Groups with Small Conjugacy Classes
Yean Nee Tan, Guan Aun How and Miin Huey Ang 244

An Explicit Basis on Riemann-Roch Space of Elliptic Function Fields
Yean Nee Tan, Ti-Chung Lee and Miin Huey Ang 252

$\delta\theta g$-closed Fuzzy Sets and its Applications
A. H. Zakari 267

Contributed Talks : Statistics / Applied Statistics / Financial Mathematics / Operations Research 280

Adaptive Blind Random Search
Maria Eda B. Arado, Emily Amor A. Balase and Roberto N. Padua 280

Configuration Change Test for Serial Contingency Tables
Emily Amor A. Balase and Roberto N. Padua 295

Optimization of Dynamic Characteristics using Genetic Algorithms
Nicolo Belavendram 306

Optimization of Dynamic Characteristics with Variable Objective Functions
Nicolo Belavendram 317

Fuzzy Superfluous Submodule
D. K. Basnet, N. K. Sarma and L. B. Singh 330

Measuring Online Bank Profit Efficiency: A stochastic frontier analysis
Md. Azizul Baten and Anton Abdulbasah Kamil 336

SIR Epidemic Model with Varrying Total Population Size
Dumrongpokaphan T., Kaewkheaw T., and Ouncharoen R. 351

Comparing Models for Fitting Zero-inflated Data
Manad Khamkong 362

Selecting among Families of Lifetime Distributions
Prasong Kitidamrongsuk and Pachitjanut Siripanich 367

Optimal Approach of Many Linear Objects to One
Atamurat Kuchkarov, Gafurjan Ibragimov and Marzieh Khakestari 377

A Simple Crank Nicolson Scheme for Asian Option
Tse Yueng Lee and Seong Tah Chin 381

Recent Advancements of Nurse Scheduling Models and a Potential Path
Huai Tein Lim and Razamin Ramli 395

The Mean Difference between Two Populations for Bernoulli and Normal Covariate Distributions
Marzuki Abubakar 410

Maximum Likelihood Estimation of Parameters in Tobit-Piecewise Regression Model
Titirut Mekbunditkul and Pachitjanut Siripanich 418

Re-weighted Robust Control Charts for Individual Observations
Mandana Mohammadi, Habshah Midi and Jayanthi Arasan 426

The Relationships of Real Estates and Stock Markets in Asia
Abdul Halim B Mohd Nawawi, Nurul Nisa' Khairol Azmi and Futeri Jazeilya Md Fadzil 436

Exchange Rates: A Comparison with Robust Regression Approach
L. Muhammad Safiih and D.A Anthea 449

Linear Programming for Parking Slot Optimization: A Case Study at Jl. T. Panglima Polem Banda Aceh
Said Munzir, Mahyus Ikhsan and Zainal Amin 462

Estimation of Population Mean in Two Phase Sampling using Attribute Auxiliary Information
Nadeem Shafique Butt and Muhammad Qaiser Shahbaz 473

A Comparative Study of Maximum Likelihood and Bayesian Estimation Approaches in Estimating Frailty Mixture Survival Model Parameters
Oh Yit Leng and Zarina Mohd Khalid 478

A Bound on the Thrifty Policy for Non-Preemptive Processing of Jobs with Increasing Cost
Katrina Gabrielle Padua and Marrick Neri 493

Comparative Performance of Two Parametric Families of Universal Portfolios
Sook Theng Pang and Choon Peng Tan 503

Multiple Correspondence Analysis on Public Service in Sabang Tourism Area
Evi Ramadhani, Devi Susanti, Asep Rusyana and Nazaruddin . . . 515

A Proposed Model of a Microcredit Institution: Break-Even Analysis, Borrowing Group Creditworthiness and Risk Analysis
Debalina Roy and Koushik Ghosh 524

Linear Programming and Sensitivity Analysis for Optimizing Nutrient Sufficiency
Asep Rusyana, Dewi Susanti, Evi Ramadhani and Nazaruddin . . . 537

A Game Theory Framework for Clustering
Abdellah Salhi, Berthold Lausen, Fajriyah Rohmatul, Marwa Baeshen, and Özgün Töreyen 552

Comparison Traditional and Model Assisted Estimators in Inverse Sampling with Replacement
Sureeporn Sungsuwan and Prachoom Suwattee 565

Performance of the Helmbold Universal Portfolio to the Initial Starting Portfolio
Choon Peng Tan and Wei Xiang Lim 577

Categorical Data Analysis on Labor Force Data in Malaysia
Sin Yin Tan, Yue Fang Loh, Aminah Bte Ahmad and Nithyaroobini A/P Munian 587

Can The Effects of Using All Suitable Lags of an Instrument Comparable to that of an Additional Basic Instrument In Instrumental Variable Regresssion Analysis of Heteroskedastic Linear Models?
Chee Yin Yip, Hock Eam Lim, Pei Yee Hong and Seng Joe Yip 598

Revisit Problems Encountered in Linear Regression Models
Chee Yin Yip and Hock Eam Lim 609

Survey of Notebook in Tertiary Education using Confounded Factorial CCE Method
Chin Khian Yong and How Hui Liew 620

Parameter Estimation of the SIR Model using the Multistage Adomian Decomposition Method (MADM)
Nuraini Yusoff, Harun Budin and Salemah Ismail 635

A Stationarity Test on Markov Chain Models based on Marginal Distribution
Mahboobeh Zangeneh Sirdari, M. Ataharul Islam, and Norhashidah Awang 646

Contributed Talks : Applied Mathematics / Image Processing / Computer Science 659

Embedding using Spread Spectrum Image Steganography with $GF^{(2m)}$
Suhaila Abd Halim and Muhammad Faiz Abdullah Sani 659

A Novel Double Stage Dynamic Time Warping Algorithm for Image Template Matching
Somya Adwan and Hamzah Arof 667

Integer Programming Model for Operational Aircraft Maintenance Routing Problem with Side Constraints
Suaibatul Aslamiah, Siti R.Simamora, Tan Kim Hek, Novin M.Sarina, Edi L.Harahap, Malem Karina 677

Stochastic Programming Model for Production Planning of Fish Processed Products
Ana Uzla Batubara, Eri Saputra, Herman Mawengkang 690

The Shortest Path between Two Points on some Surfaces by using the Application of Euler Equation
Nathaphon Boonnam and Pakkinee Chitsakul 704

Solving Nonlinear Algebraic Equation by Homotopy Analysis Method
Chin Fung Yuen, Lem Kong Hoong and Chong Fook Seng 712

An Alternative Homotopy Analysis Method in Solving Differential Equation under Finite Order of Deformation
Chong Fook Seng, Lem Kong Hoong and Chin Fung Yuen 721

A Modified Algorithm For The Homotopy Perturbation Method With Applications To Lotka-Volterra Systems
M. S. H. Chowdhury, T. H. Hassan and A. F. Ismail 731

Numerical Solution of Nonlinear Fredholm-Volterra Integro-differential Equations using Legendre Wavelets
M. Dadkhah, M. Tavassoli Kajani and S. Mahdavi 738

Development of Labor Force Condition in Solow Economic Growth Model
Open Darnius 745

Generalized Space-Time Autoregressive Modeling
Dhoriva Urwatul Wutsqa, Suhartono and Brodjol Sutijo 752

Scenario-based Approach for Ranking DMUs in Stochastic DEA Model
Syahril Effendi 762

The Implementation of the Stochastic Progamming model for River Water Quality Management
Adelina Harahap, Muda M.Ginting, Sopar Siregar, Muhammad Nur Eddy, Lesman Tarigan, Herman Mawengkang 772

An Optimization Model for Water Resources Management under Uncertainty
Indriyani, Tohom P. Banjarnahor, Nelson Nababan,Yusleni, Atur H.Samosir, Herman Mawengkang 786

Effective Neurospora Process Model on Light and FRQ Protein
Kanchana Kumnungkit and Sarawut Suwannaut 796

Neurospora Biorhythm Mathematical Model with Light-Dark Cycle
Kanchana Kumnungkit and Nipon Wongvisetsirikul 809

Variational Iteration Method for Euler Differential Equation
Wuryansari Muharini Kusumawinahyu 822

An Optimization Model for Sustainable Forest Management to Preserve Water Allocation for Hydroelectric Power Plant
Erna Laily, Gevoner Harianjak, Nilawati, R.Harahap, Bistok Purba, Herman Mawengkang 834

A Numerical Study of Ships Rolling Motion
How Hui Liew and Yean Fong Pan 843

Exit Selection by Occupant During Building Evacuation using Neural Network
Eng Aik Lim 852

Mathematical Model for Analyzing the Value of Cooperational Leadership Based on Multi Agent System
Abil Mansyur, Elmanani Simamora 865

Solving Systems of Nonlinear Equations Based on Constrained Search Approach
Mardiningsih 886

A Goal Programming Model for the Recycling Supply Chain Problem
Putri K.Nasution,Rima Aprilia,Amalia,Herman Mawengkang . . . 903

An Active Set Method with Central Measure on Removing Impulse Noise
Marrick Neri . . . 917

Inner Solution for Oscillatory Free Convection about a Sphere Embedded in a Porous Medium
Lai Zhe Phooi, Rozaini Roslan, Ishak Hashim, and Zainodin Haji Jubok . . . 926

The use of Adomian Decomposition Method for Solving Generalised Riccati Differential Equations
T.R. Ramesh Rao . . . 935

Subclasses Discriminant Analysis by Fuzzy Cluster Algorithm
Ghasem Rekabdar, Naser Haddadzadeh and Davood Seifipoor . . . 942

A Multi-stage Stochastic Optimization Model for Water Resources Management
Elly Rosmaini . . . 951

Stochastic Programming Model for Land Management Problems
Siti Rusdiana . . . 962

Predator-prey Model in a Bioreactor with Death Coefficient
Zubaidah Sadikin and Normah Salim . . . 973

Recommending a Hybrid Method for Solving the Ordered Crossover Problem
Bahador Saket and Farnaz Behrang . . . 984

Solving Integer Goal Programming Problems Based on a Reference Direction Algorithm
Sawaluddin . . . 1000

Integer Programming Model for Supply Chain with Market Selection
Selamat Siregar, Agusman, Sindak Situmorang, Lisbet Marbun, Abdul Jalil, Herman Mawengkang . . . 1012

Automatic Gridding for DNA Microarray Image using Image Projection Profile
Joko Siswantoro . . . 1028

Efficient Reduction of Fuzzy Finite Tree Automata
Somaye Moghari, Mohammad Mehdi Zahedi and Reza Ameri . . . 1034

Minimization of Fuzzy Finite Tree Automata
Somaye Moghari, Mohammad Mehdi Zahedi and Reza Ameri . . . 1044

An Improved Strategy for Solving Quadratic Assignment Problems
Susiana, Nunik Ardiana, Wahab Y.S.Hasibuan, Herman Mawengkang 1053

An Optimization Model for Multi-echelon Supply Chain Planning with Reliability Consideration
Suyanto 1061

An Algorithm Based on Direct Search Approach for Solving Mixed-Integer Nonlinear Programming Problems
Astri Syafrianty, Nenna I.Syahputri, Meilinda Siahaan, Herman Mawengkang 1082

Dynamic Properties of an Aggregate Econometric Model of Indonesias Economy
Intan Syahrini 1092

A Direct Search Algorithm for Solving the Multi-period Single-sourcing Problem
Miduk Tampubolon, Vita Damayanti, Herman Mawengkang 1101

An Optimization Model for Cargo Container Loading Problems under Uncertainty
Davidson Tarigan,Nurelista Dahyaruci,Rusli Tarigan, Herman Mawengkang 1116

Comparisons of Effects for Reducing Geosmin Tainted Off-Flavor and Physical Qualities in Frozen Thai Panga (Pangasius sp.) Fish Fillets
Piyavit Thipbharos 1135

A Mixed Integer Nonlinear Stochastic Programming Model in Tackling a Superstructure Synthesis Water Networks Optimization Problem with Uncertainty Parameter
Eriek M.L.Tobing, Eva Y.Siregar, Mizan, Herman Mawengkang . . 1145

Optimal Control for SEIR Rabies Model between Dogs and Human with Vaccination Effect in dogs
Eti Dwi Wiraningsih, Widodo, Lina Aryati, Syamsuddin Toaha and Suzanne Lenhart 1161

A Solitary-like Wave Generated by Flow Passing a Bump
Leo Wiryanto 1176

Titles of Poster Presentations 1185

Organising Committee 1186

Acknowledgment 1187

Automatic Gridding for DNA Microarray Image Using Image Projection Profile

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Abstract. DNA microarray is powerful tool and widely used in many areas. DNA microarray is produced from control and test tissue sample cDNAs, which are labeled with two different fluorescent dyes. After hybridization using a laser scanner, microarray images are obtained. Image analysis play an important role in extracting fluorescence intensity from microarray image. First step in microarray image analysis is addressing, that is finding areas in the image on which contain one spot using gird lines. This step can be done by either manually or automatically. In this paper we propose an efficient and simple automatic gridding for microarray image analysis using image projection profile, base on fact that microarray image has local minimum and maximum intensity at background and foreground areas respectively. Grid lines are obtained by finding local minimum of vertical and horizontal projection profile. This algorithm has been implemented in MATLAB and tested with several microarray images.

1 Introduction

DNA microarray is powerful tool and widely used in many areas, e.g. for human genetic research and drug discovery. DNA microarray is produced from control and test tissue sample cDNAs, which are labeled with two different fluorescent dyes, usually the red fluorescent dye Cy5 for the control and green fluorescent dye Cy3 for the test and then printed on a glass microslide containing gene specific cDNA clones arranged in an array format for hybridization. After hybridization using a laser scanner, images of the microarray are obtained [1].

Image processing and analysis play an important role in extracting fluorescence intensity from microarray image. there are The three basic steps of microarray analysis are:

1. Addressing: finding areas in the image on which contain one spot using gird lines. The combined area of the spot and its background is called the target area.
2. Segmentation: portioning the target area of every spots into two distinct regions, foreground (the spot its self) and background.

3. Reduction: extracting two scalar values for the intensity of *R* and *G* and assigning one value *R*/*G* for relative abundance to each spot.

The first step can be done by either manual grid alignment or fully automated (automatic girdding) [5].

Fig. 1. Microarray

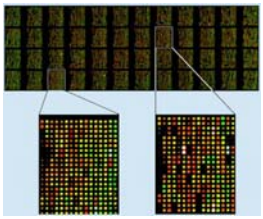

Fig. 2. Microarray image

Many available tools provide algorithms for microarry image analisys, among these, GenePix [3], Imagene [4], QuantArray [7] and ScanAlyze [6] are widely used. Most methods assume circular spot shapes and require manual alignment of the grid locations. Therefore, automated grid and spot finding are highly desirable. The Gridclus algorithm is an algorithm for microarray image automatic gridding base on k-mean clustering has been presented [5], but this algorithm is not efficient in time computing [12]. Another algorithm uses mathematical morphology, including image projection profile and other complex mathematical morphology operations [9]. In this paper we propose an efficient and simple automatic gridding for microarray image analysis using image projection profile, base on fact that microarray image has local minimum and maximum intensity at background and foreground areas respectively.

2 Mathematical Backgrounds

In this section, we provide some mathematical backgrounds which are used in microarray image automatics gridding. Suppose $\mathbb{Z}$ is the set of integers, E is a non-empty and finite subset of $\mathbb{Z}$ and K is an interval $[0,k]$ of $\mathbb{Z}$, with $k>0$. A function f from E to K, $f \in K^E$, represents a gray-scale image. A pixel is an element of E, $p \in E$ is a point in an image f and its gray-level is $f(p)$.

Definition 1. The union of two gray-scale images f_1 and f_2, denoted by $f_1 \vee f_2$, is the function in K^E defined as, for any $p \in E$, $(f_1 \vee f_2)(p) = \max\{f_1(p), f_2(p)\}$.

Definition 2. For a pixel $(a,b) \in E$, $E_{x=a} \subset E$ is the cutting line E in vertical direction at (a,b) defined as $E_{x=a} = \{(a,y) \in E\}$. Similarly, $E_{y=b} \subset E$ is the cutting line E in horizontal direction at (a,b) defined as $E_{y=b} = \{(x,b) \in E\}$.

Definition 3. Suppose $f : E \to K$ is a gray-scale image, the horizontal projection profile of *f*, denoted by $P_h(f)$, is a function from $E_{x=0}$ to $\mathbb{Z}$ defined as $P_h(f)(0,b) = \sum_{p \in E_{y=b}} f(p)$, for any $(0,b) \in E_{x=0}$. The vertical projection profile of *f*, denoted by $P_v(f)$, is a function from $E_{y=0}$ to $\mathbb{Z}$ defined as $P_v(f)(a,0) = \sum_{p \in E_{x=a}} f(p)$, for any $(a,0) \in E_{y=0}$.

Definition 4. $P_h(f)$ has local maximum (resp. local minimum) at $(0,b_0)$ if $P_h(f)(0,b_0) \geq P_h(f)(0,b)$ (resp. $P_h(f)(0,b_0) \leq P_h(f)(0,b)$) for any $(0,b)$ in some neighborhood of $(0,b_0)$. $P_v(f)$ has local maximum (resp. local minimum) at $(a_0,0)$ if $P_v(f)(a_0,0) \geq P_v(f)(a,0)$ (resp. $P_v(f)(a_0,0) \leq P_v(f)(a,0)$) for any $(a,0)$ in some neighborhood of $(a_0,0)$.

3 Automatic Gridding

Two matrix of intensity values are extracted from microarray image, one for the red channel and one for the green channel. Automatic gridding algorithm with projection profile is started with defining $R(i,j)$ and $G(i,j)$ as intensity values for the red and the green channel, for $i = 1,2,\ldots,M$, $j = 1,2,\ldots,N$, where *M* and *N* are the total number of pixel rows and columns respectively, on entire microarray image. The next steps for automatic gridding are:

1. Define *f* as union of *R* and *G*. the output is matrix $\mathbf{f}$.
2. Calculate horizontal projection profile $P_h(f)$ and vertical projection profile $P_v(f)$ for *f*. The output are vectors $\mathbf{p}_h$ and $\mathbf{p}_v$.

3. Determine locations of local minimum for $P_h(f)$ and $P_v(f)$. The output are vectors $\mathbf{m}_h$ and $\mathbf{m}_v$.
4. Determine locations of local maximum for $P_h(f)$ (resp. $P_v(f)$). To avoid local maximum in background area choose local maximum such that its value less than $\max\{\mathbf{p}_h\}/c$ (resp. $\max\{\mathbf{p}_v\}/c$) for some integer $c>0$ (e.g. $c=3$). The output are vectors $\mathbf{M}_h$ and $\mathbf{M}_v$.
5. Between each pair of contiguous element of $\mathbf{M}_h$ (resp. $\mathbf{M}_v$), determine smallest and largest elements of $\mathbf{m}_h$ (resp. $\mathbf{m}_v$). Calculate average of such as smallest and largest elements of $\mathbf{m}_h$ (resp. $\mathbf{m}_v$) as row (resp. column) gird lines.

This algorithm has been implemented in MATLAB and tested with several microarray images one of them is microarray image with resolution 531×533 pixels and contain 14×14 microarray spots as shown in figure 3. Figure 4 show union of figure 3, figure 5 and 6 show horizontal and vertical projection profiles of image shown in figure 4.

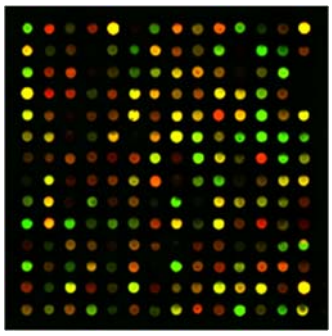

Fig. 3. Microarray image used in implementation

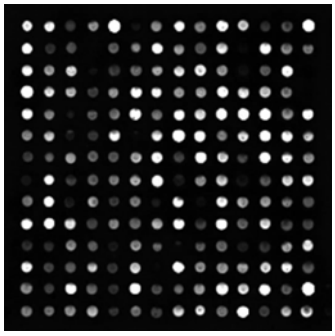

Fig. 4. Union of *R* and *G*

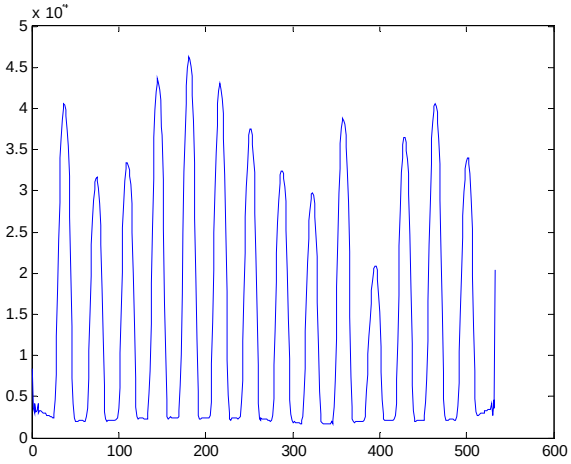

Fig. 5. Horizontal projection profile

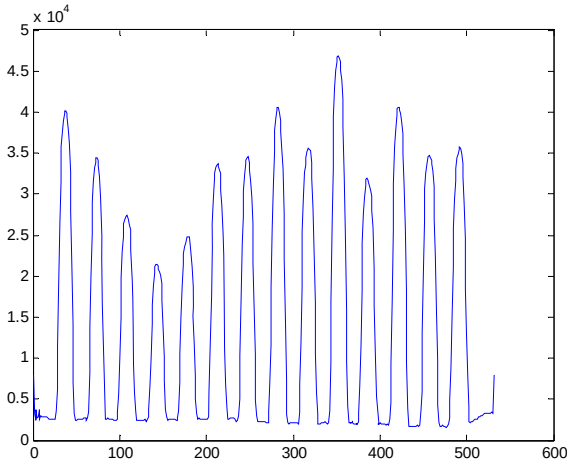

Fig. 6. Vertical projection profile

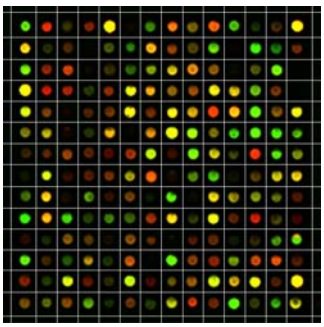

Fig. 7. Result of automatic gridding

The result of automatic gridding algorithm using projection profile for figure 3 are 15 horizontal and 15 vertical line as shown in figure 7. This algorithm is efficient in time computing, in MATLAB this algorithm needs approximately 8 until 9 second for computing automatic gridding for figure 3.

3 Conclusion

First step in microarray image analysis is addressing, that is finding areas in the image on which contain one spot using gird lines. This step is one of the most important step in microarray image analysis and can be done by either manually or automatically. The Gridclus algorithm is an algorithm for microarray image automatic gridding base on k-mean clustering has been presented, but this algorithm is not efficient in time computing. Another algorithm uses mathematical morphology, including image projection profile and other complex mathematical morphology operations. An efficient and simple automatic gridding for microarray image analysis using image projection profile has introduced. This algorithm base on fact that microarray image has local minimum and maximum intensity at background and foreground areas respectively.

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