Classification of Micro Array Protein Crystal Images With Laplacian Pyramidal Filters and Neural Networks

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INTRODUCTION

- This project is part of the Northeast Structural Genomics Consortium (NESG). The goal of this consortium is to develop efficient and integrated technologies for high-throughput (HTP) protein production and 3D structure determination.
- This project focuses on the design of an image analysis system to classify protein crystal structures in a production oriented environment.
- The method presented performs classification of microscopic images as protein precipitates or crystals versus clear solutions.
- Preliminary experiments are presented, which provided high classification accuracy on large datasets of protein crystallization experiments using expert classification for ground truth.

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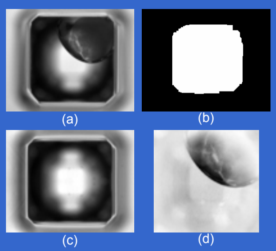
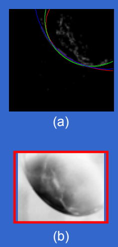
METHODOLOGY

1. Preprocessing of Microscopic Images



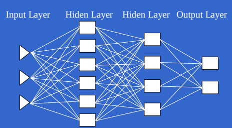
(a) Original image with an oil drop containing a precipitate. (b) Mask computed from the contours of the wells. (c) Background image acquired without drops. (d) Pre-filtered image.



(a) Ellispoidal Hough transform [1] to detect the three most probable ellipses, plotted over the edge map of the pre-filtered image. (b) Pre-filtered image cropped with the minimal rectangular area encompassing the three ellipses.

2. Feature Extraction with Laplacian Pyramidal Expansion [2]

3. Classification with a Feed-Forward Neural Network



Features of the Neural Network: Normalized 10-bins histograms of the Laplacian expansion coefficients from the four first detail levels.

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REFERENCES

[1] D. H. Ballard, "Generalizing the Hough transform to detect arbitrary shapes," *Pattern Recognition*, vol. 13, pp. 111-122, 1981.
[2] P. J. Burt and E. H. Adelson, "The Laplacian pyramid as a compact image code," *IEEE Transactions on Communications*, vol. 31, pp. 532-540, 1983.
[3] C. A. Cumbaa, A. Lauricella, N. Fehrman, C. Veatch, R. Collins, J. Luft, G. DeTitta, and I. Jurisica, "Automatic classification of sub-microlitre protein-crystallization trials in 1536-well plates," *Acta Crystallographica Section D-Biological Crystallography*, vol. 59, pp. 1619-1627, 2003.
[4] I. Jurisica, P. Rogers, J. I. Glasgow, R. J. Collins, J. R. Wolfley, J. R. Luft, and G. T. DeTitta, "Improving objectivity and scalability in protein crystallization," *IEEE Intelligent Systems*, vol. 16, pp. 26-34, 2001.

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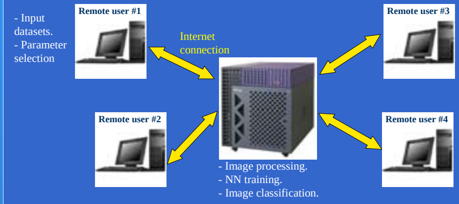
METHODOLOGY

Remote Application Server: Web page interface managed by a Matlab© Server.



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DATABASES

1. Microscopic images were acquired with a CCD camera under robotic control.
2. Gray scale 8-bits images saved in tiff/jpeg format.
3. Arrays of 256 $\times$256 wells screened at three time instants after experimental setup: 1 day , 1 week and 2 weeks.
4. Database of 13,490 scored images:
   - 509 drops containing precipitates and/or crystals.
   - 3,349 clear drops.
   - 9,632 'others' combining drops with organic matter, or dye.

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INITIAL STUDY

- **Training data set**
  - 100 images
  - 1/3 clear drops, 2/3 drops with precipitates/crystals
- **Binary classification**
  - 0 = clear drop, 1 = not a clear drop.
- **Testing experiments**
  - Classification errors: false positive (FP) and false negative (FN).
    - 100 images with precipitates/crystals: FP = 24%.
    - 100 images with clear drops: FN = 28%.
  - For our application, false positive errors are more critical than false negative since it would lead the operator to discard images with precipitate/crystals.
  - ⇨ The goal of the optimization of the neural network design was therefore to minimize FP errors.
  - Performance of the classification system:
    - Accuracy = (TP+TN)/(TP+TN+FP+FN)
    - Precision = TP/(TP+FP)
    - Recall = TP/(TP+FN)

[Accuracy Precision Recall] = [74% 0.73 0.76] obtained in this study
[Accuracy Precision Recall] = [88% 0.83 0.76] [3] single plate
[Accuracy Precision Recall] = [85% 0.24 0.66] [3] multiple plates

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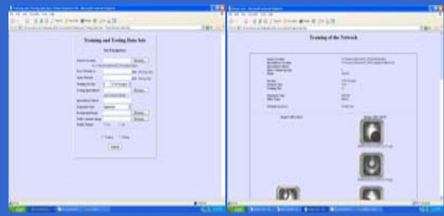
RESULTS

1. Remote Application Web-server Interface

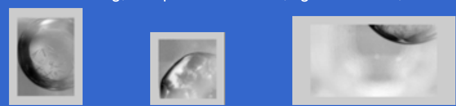


2. Classification with a New Image Database

- **Image Features for Neural Network**
  - Quantitative shape descriptions of the first-order histogram of Laplacian pyramid coefficients.
  - 8 statistics for each coefficient subset.
- **Training data set**
  - 500 images
  - 1/2 clear drops, 1/2 drops with precipitates/crystals
- **Testing experiments**
  - 100 images with precipitates/crystals: FP = 7%.
  - 100 images with clear drops: FN = 3%.

3. Examples of Misclassified Images

Motion blurring, complex boundaries, light reflection, etc.



Small bubble size, no sharp gradient, background influence, etc.

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DISCUSSION

- Introduction of robotic manipulation of the crystals for HTP protein production requires the automation of image analysis of crystallization experiments for classification of solution content.
- The proposed feed forward neural network showed promising results in classifying microscopic images.
- Features of representation were computed from Laplacian pyramid expansion histograms. The histogram made the features invariant to orientation which was a desirable feature in order to be able to characterize the diversity and complexity of precipitate appearances.
- The Laplacian expansion provided a representation of the image edge and texture patterns at different scales with extremely fast implementation.

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CONCLUSION

- Future applications of the project include further classification of the microscopic image database to separate crystals from precipitates and drops with organic matter.
- A parallel task of this project includes the creation of a web-based infrastructure for testing and development. An experimental test-bed for crystallization screening is currently being developed .

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