

Optical Scatter Imaging for Rapid Microbial Source Tracking

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Microbial Source Tracking

- Microbial source tracking (MST) is scientific pursue to determine the sources of bacteria.
- MST is important for identifying the source bacterial contamination in water and food.

Why *E. coli*?

- *E. coli* is a widely used indicator of fecal pollution in water.
- High counts of *E. coli* in water signify conditions that may pose threat to human health and force beach closures.
- To control the fecal contamination problem, it is necessary to identify the sources of contaminants.



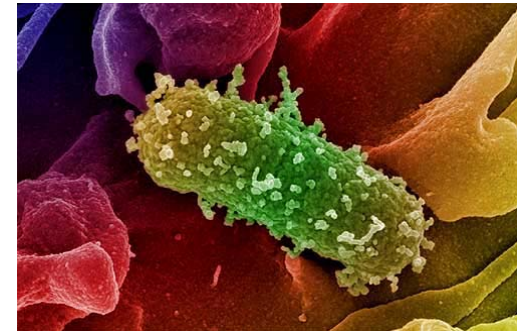
Pathogenic and Nonpathogenic *E. coli*



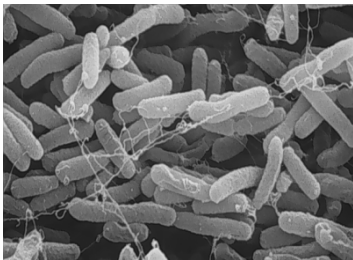
Human (Sewage, Septic tank leakage)



Farm Animals



Pathogenic *E. coli* (O157:H7)



Nonpathogenic *E. coli*



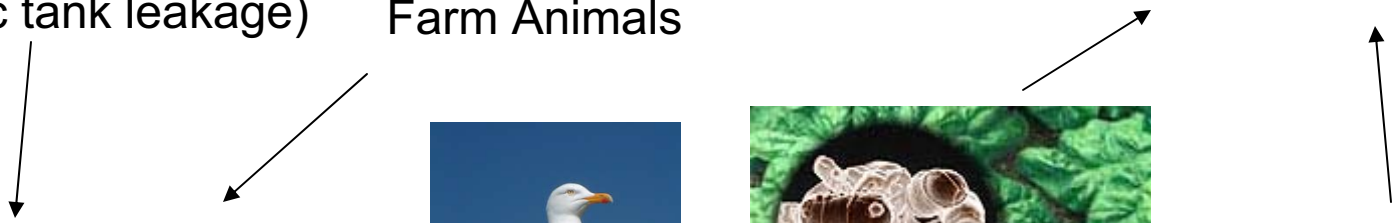
Birds/wildlife



O157:H7 contaminated food (spinach)



Raw/undercooked Beef



Existing Methods for MST

- Non-DNA based MST
- DNA based MST (DNA Typing)

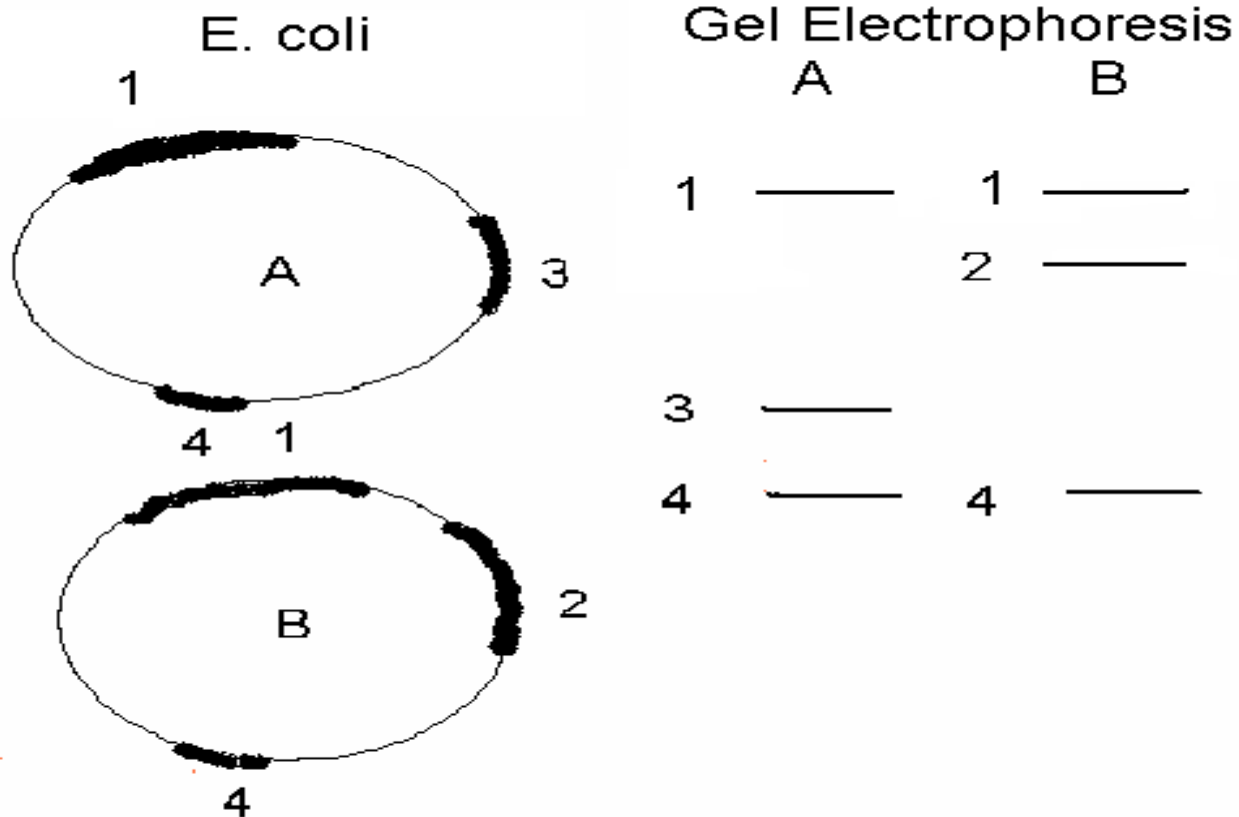
Examples of Non-DNA Based Methods

- Antibiotic resistance analysis
- Fatty acid analysis
- Optical brighteners
- Caffeine detection

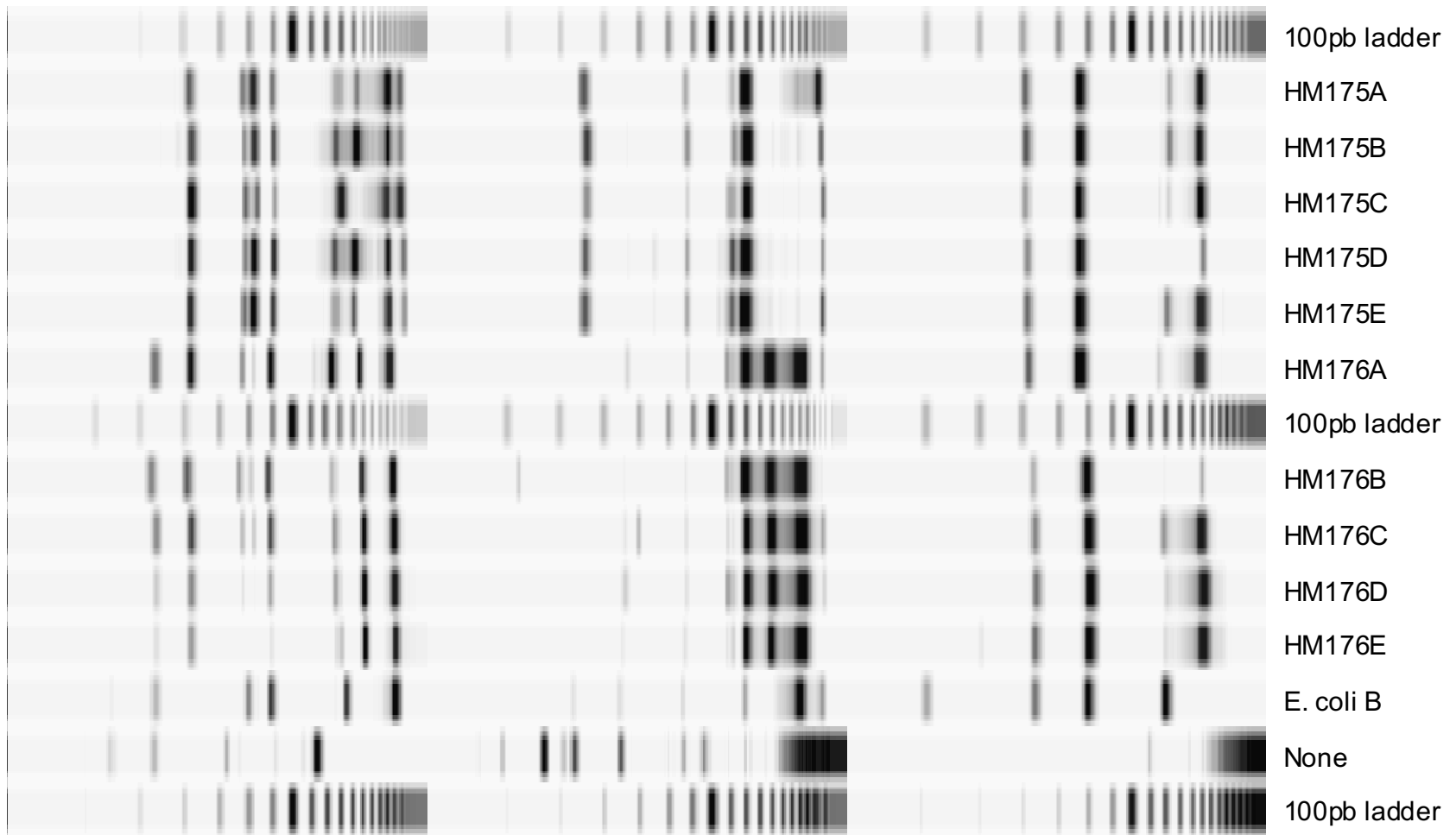
DNA Based MST Methods

- Use of PCR
 - Random amplified polymorphic DNA (RAPD)
 - PCR of repetitive intergenic sequence (Rep-PCR)
- Use of restriction enzymes
 - Pulse field gel electrophoresis (PFGE)
 - Ribotyping (RT)
 - Amplified fragment length polymorphic DNA (AFLP)

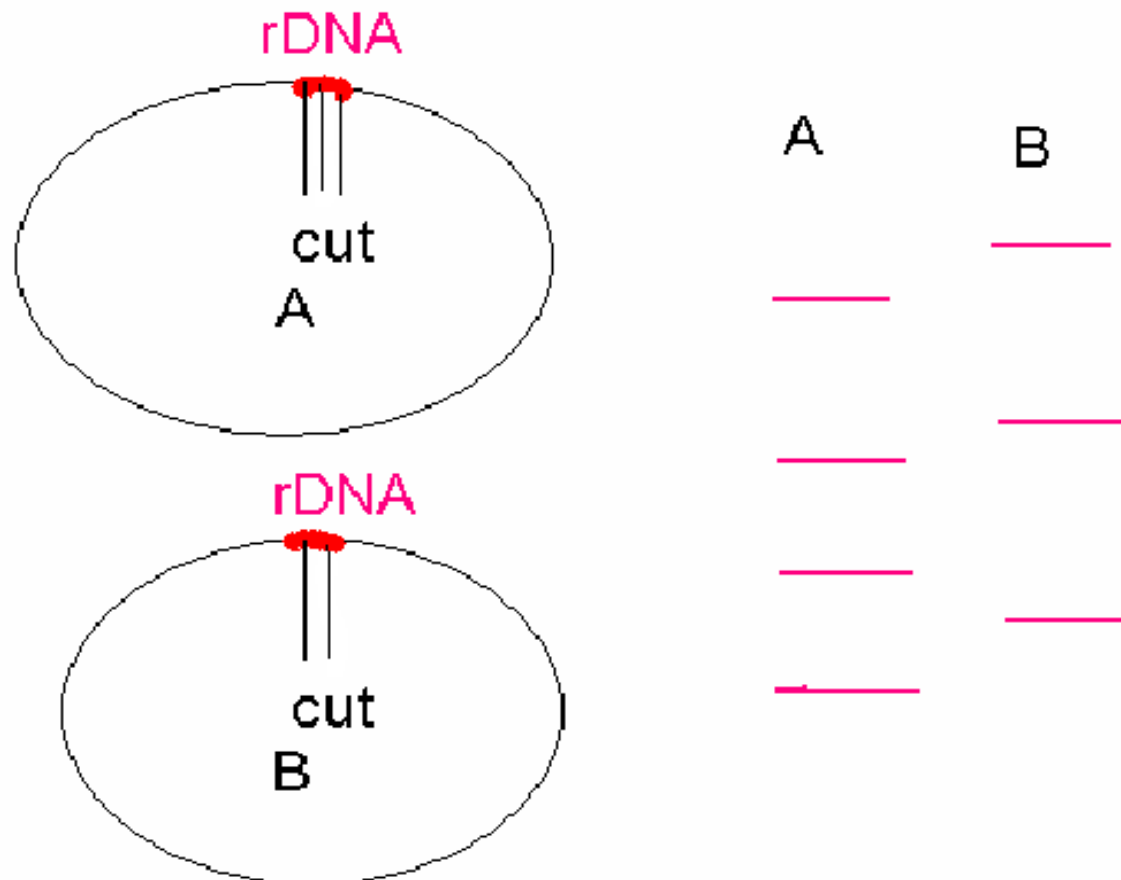
PCR-BASED DNA TYPING: RAPD or REP-PCR



E. coli RAPD DNA TYPING



Restriction Enzyme-Based DNA Typing: Ribotyping

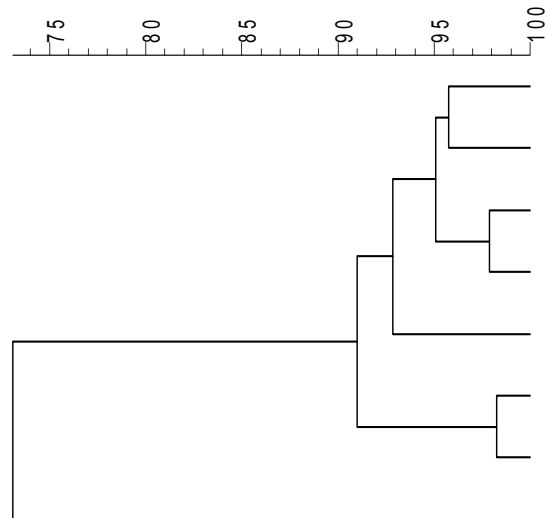


RIBOTYPING: 16S RIBOSOMAL RNA GENE (rDNA) cut with *Hind*III

Pearson correlation (Opt:1.56%) [0.0%-100.0%]

SEB1

SEB1



GS119

GS015

CW114

CW113

HM028

HS123

HS121

HM004

Genomic Based Methods

- Microarray
- Metagenomics
- Mass sequencing base SNP PCR

- These methods are thorough but require major technical efforts.

Review of New Approaches

- DNA microarray analysis
- Comparing DNA sequences of certain genes
- Metagenomics

- Use of mass sequencing based technology to identify host specific SNP PCR primers
- Laser scattering technology of host specific pattern recognition

Optical Scattering for MST

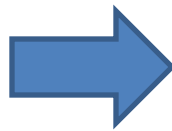
- Polarized differential light scattering for identifying bacteria above species in suspension.
- Colony based laser scattering for identification of different pathogenic bacterial species in food samples.

Major Challenges for MST within *E. coli* species:

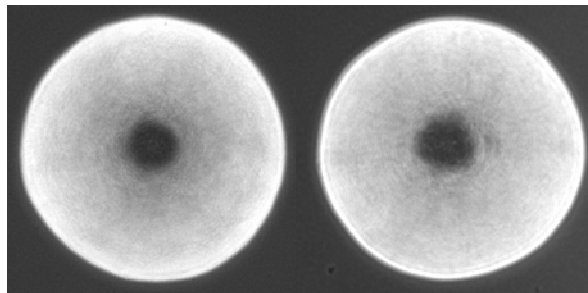
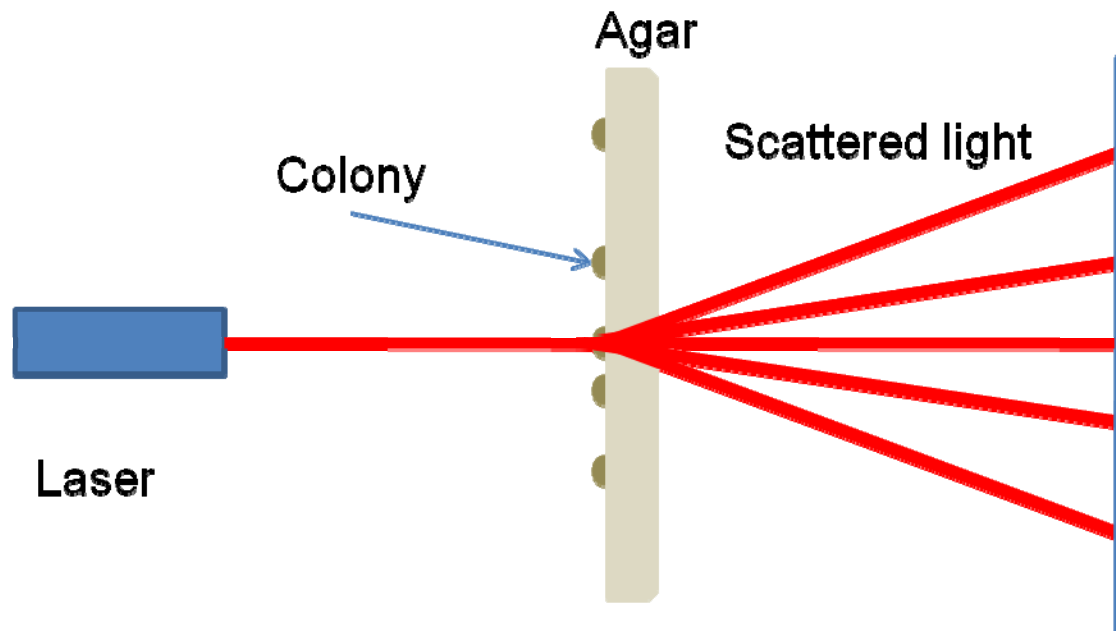
- High similarity among bacterial colonies from different hosts

Bacterial Preparation

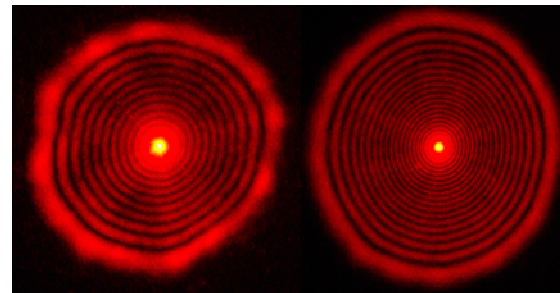
- Grow *E. coli* in broth
- Plate *E. coli* on LB agar plate
- Select colonies for laser scattering imaging



Forward Light Scattering

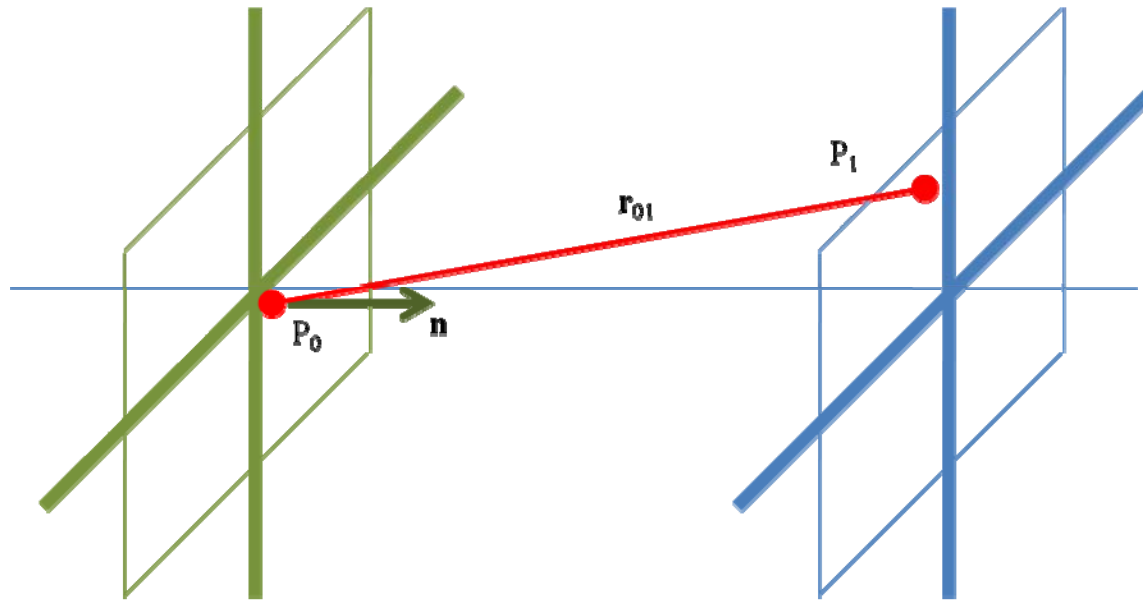


Microscopic Images



Scattering Images

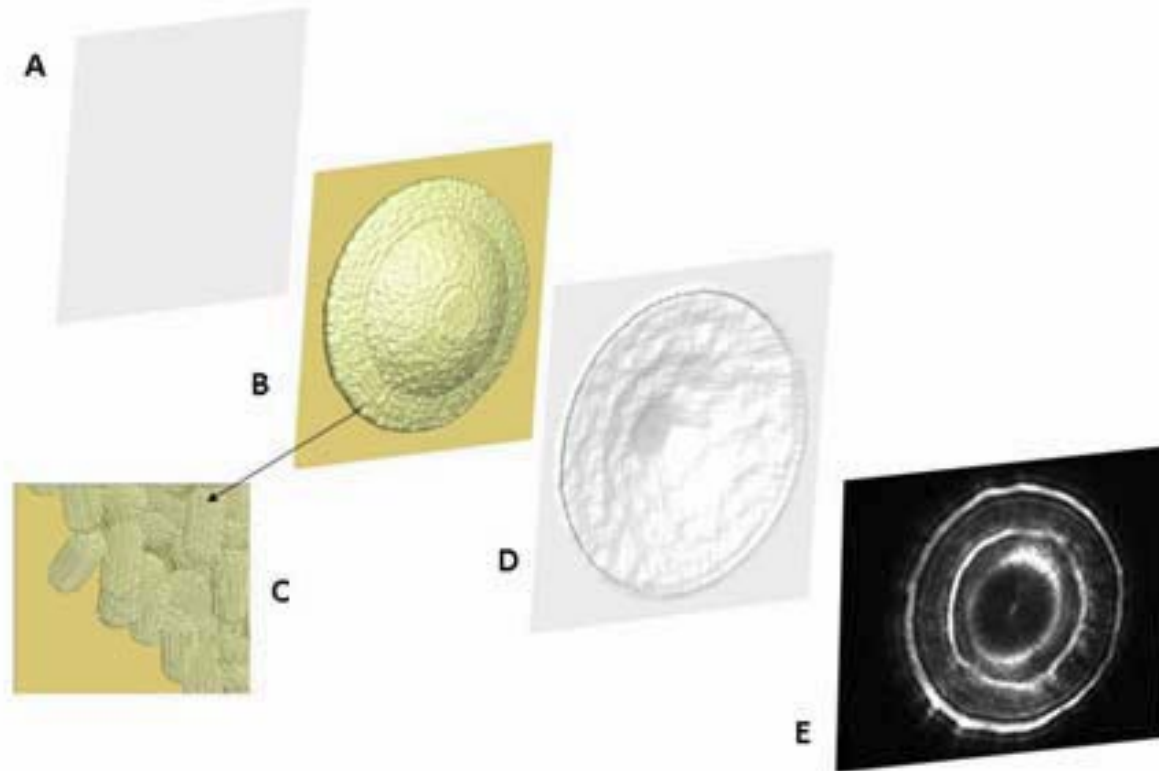
Laser Wavefront Modulation



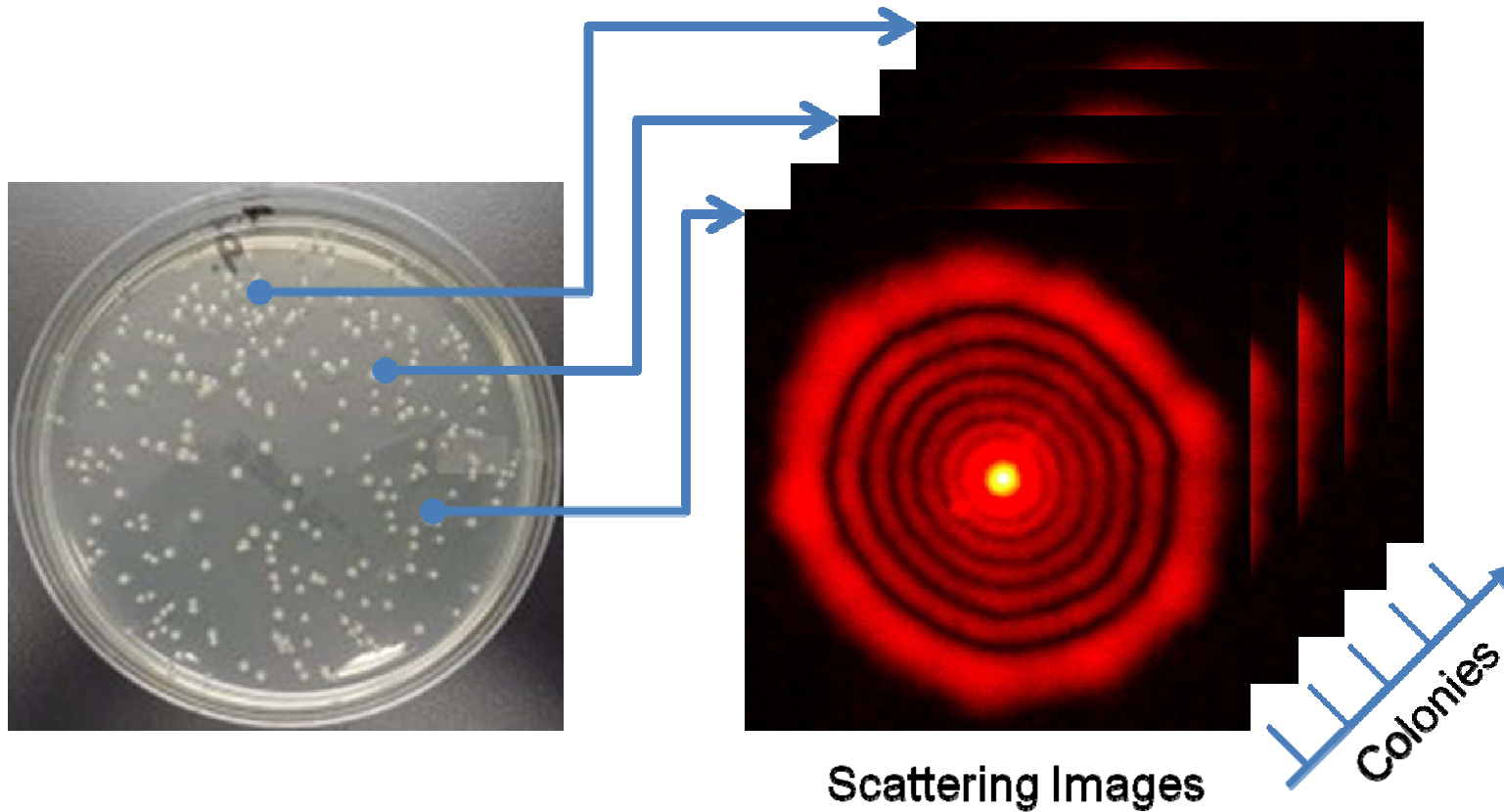
The Rayleigh-Sommerfeld diffraction formula

$$U(P_1) = \frac{1}{j\lambda} \iint U(P_0) \frac{\cos(\mathbf{n}, \mathbf{r}_{01})}{r_{01}} e^{jk r_{01}} ds$$

Laser Wavefront Modulation



Laser Scattering Images



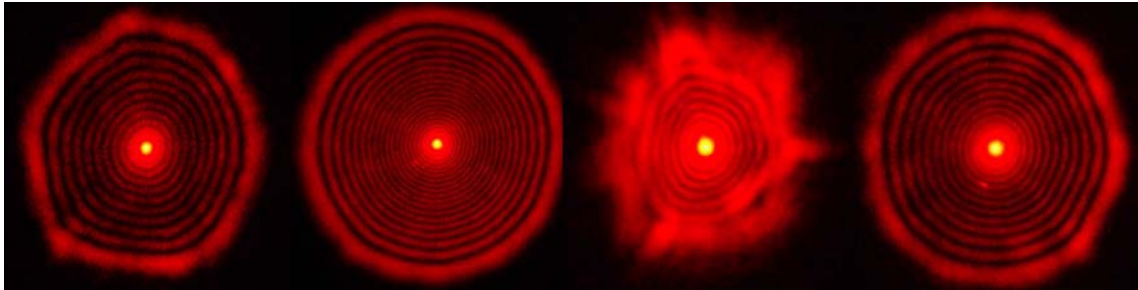
Scattering Pattern Consistency

HM63

HM04

HS148

GS125



52 days

52 days

24 days

47 days

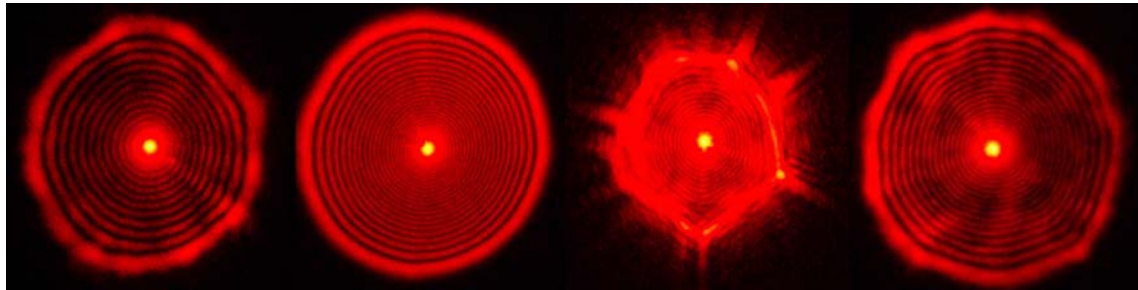
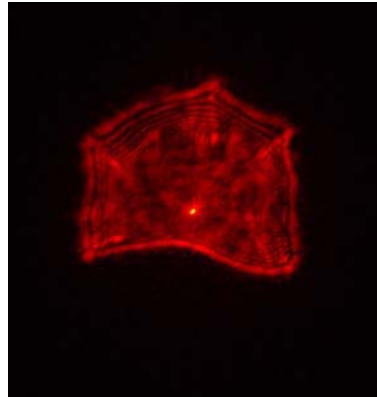
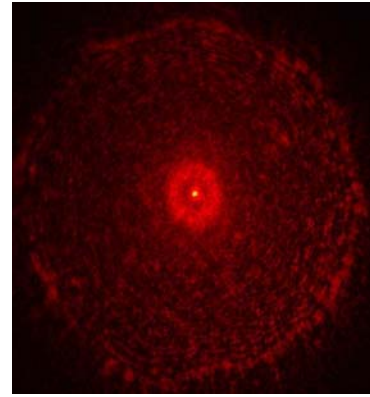


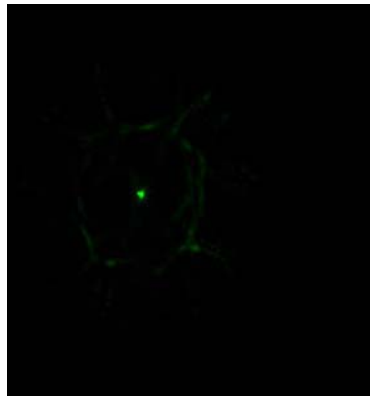
Image Selection



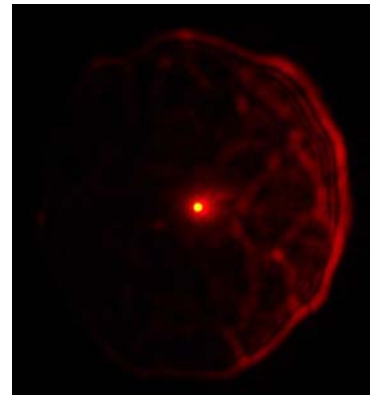
Fused colonies



Moisture

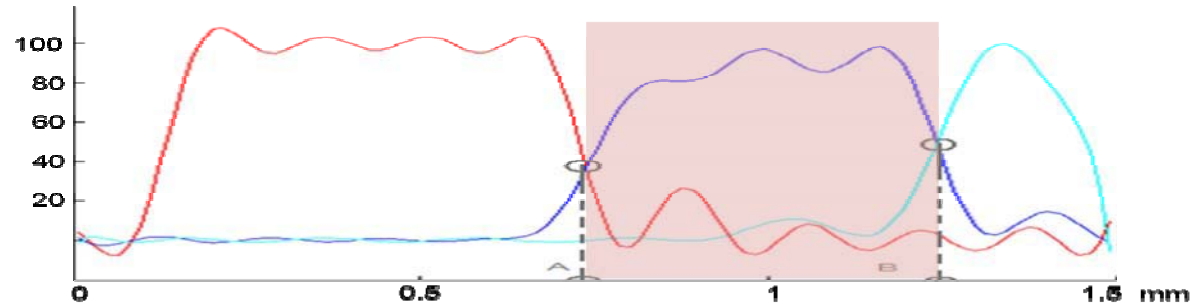


Color channels

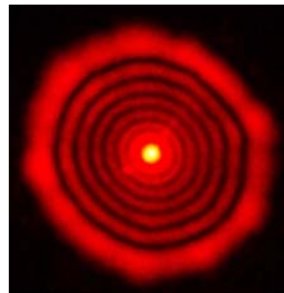


Illumination

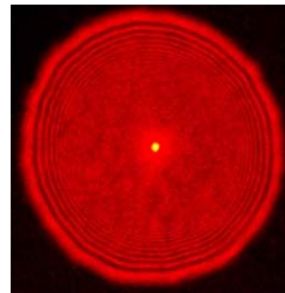
Patterns and Colony Size



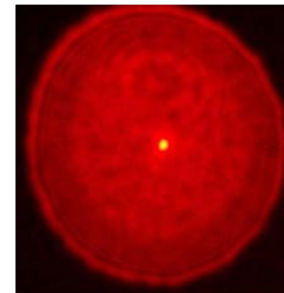
Human



0.18 mm

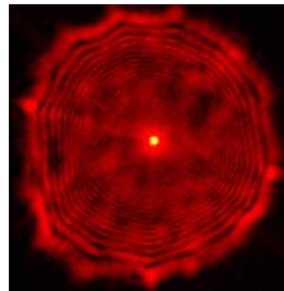


0.76 mm

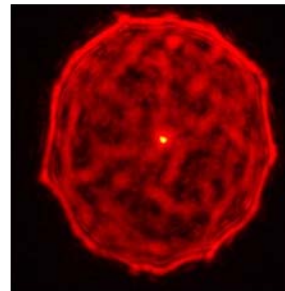


1.19 mm

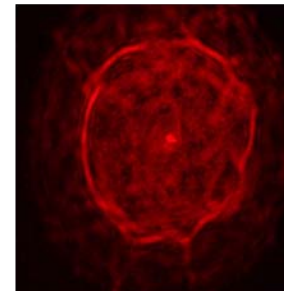
Seagull



0.58 mm

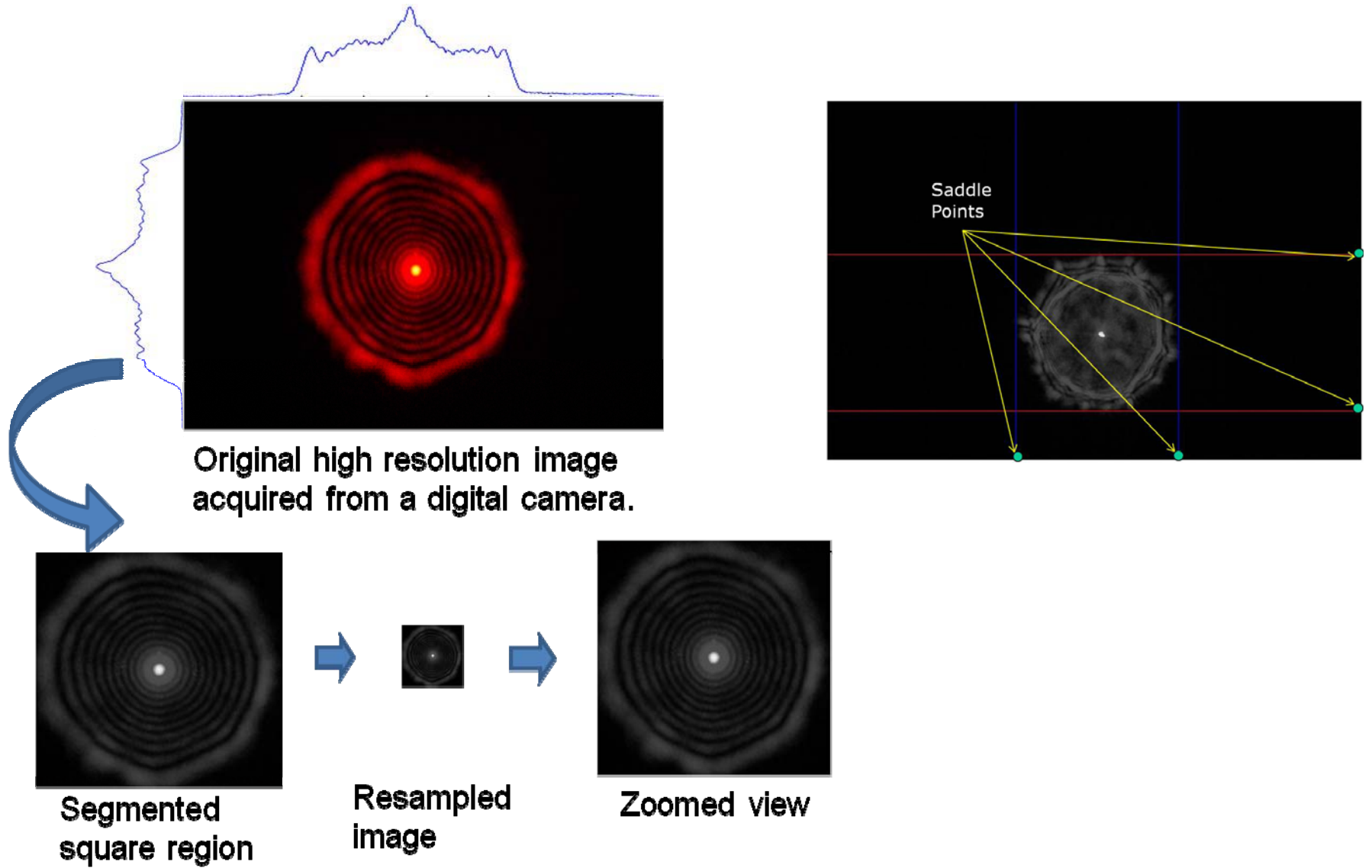


0.85 mm

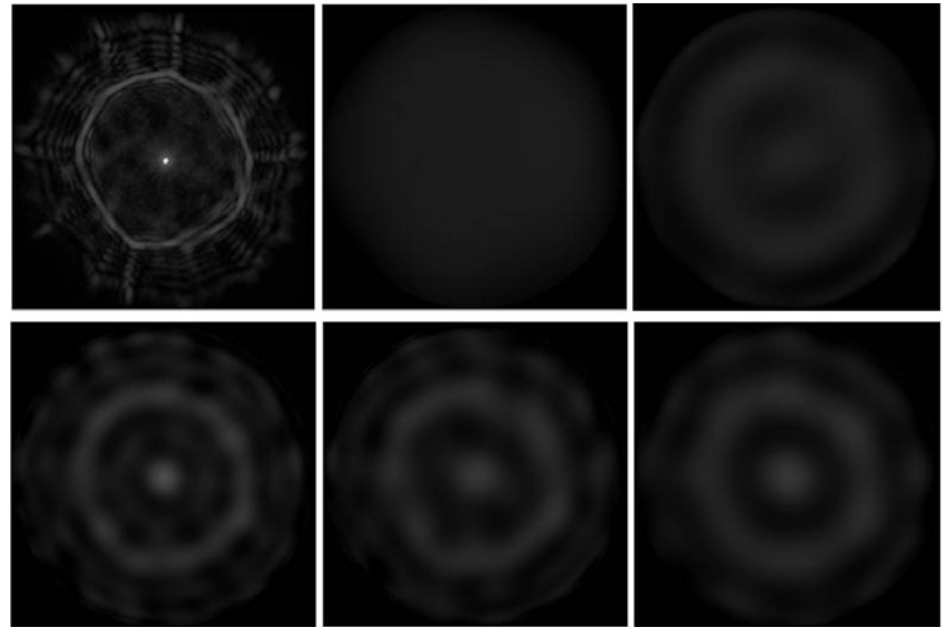
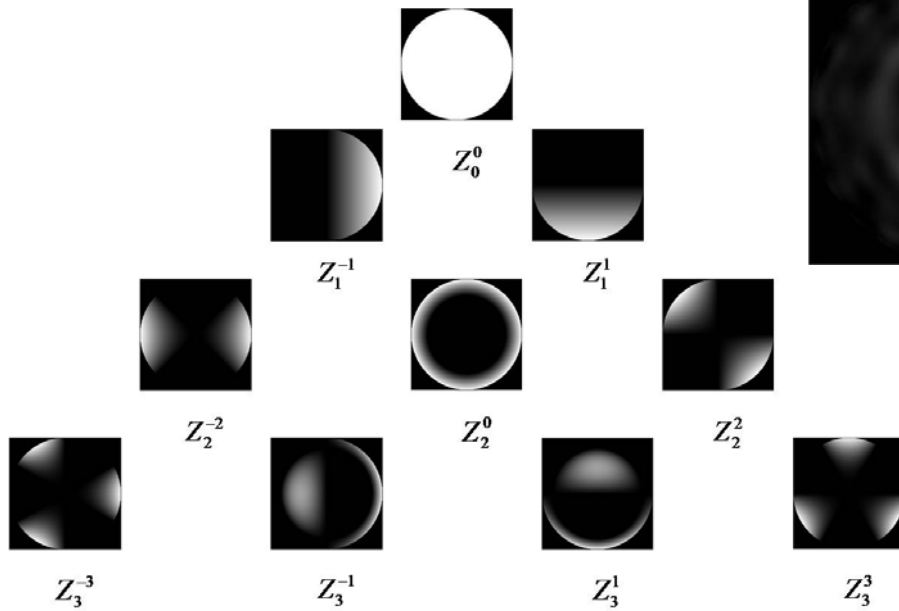


1.26 mm

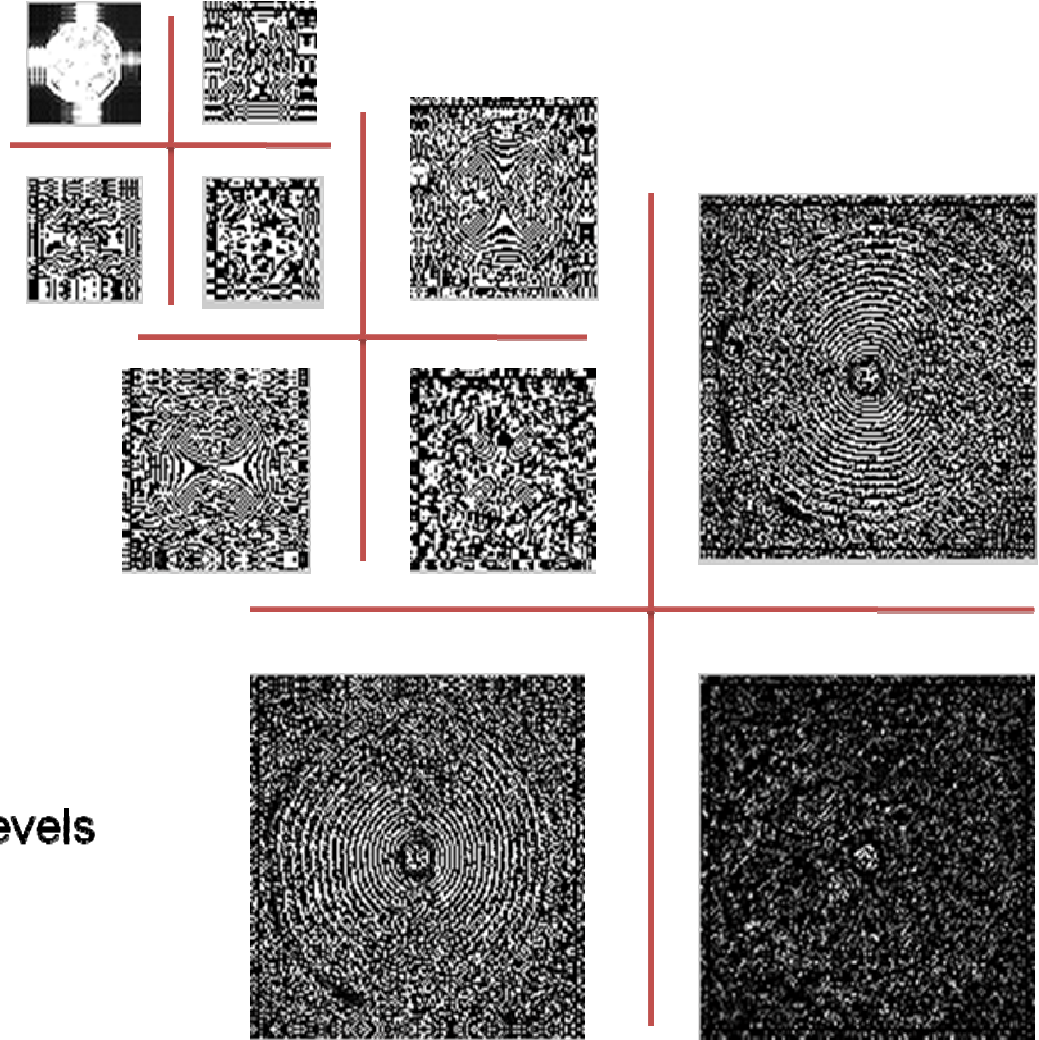
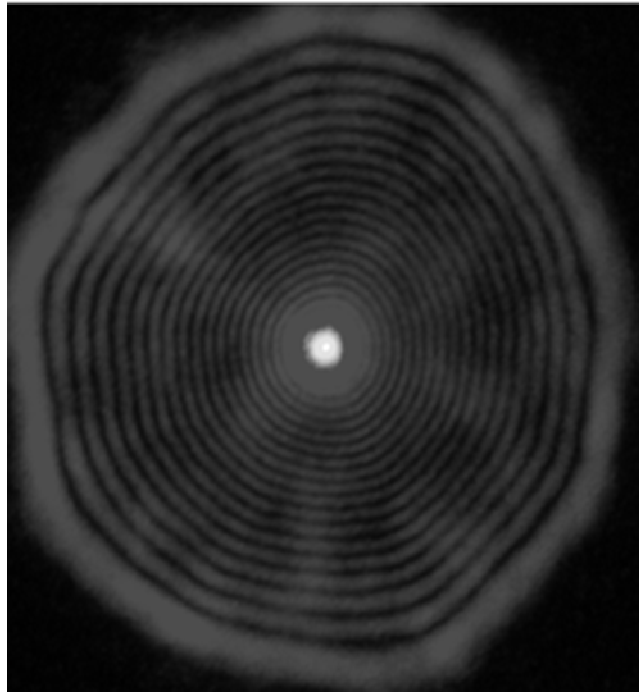
Image Preprocessing



Feature Extraction

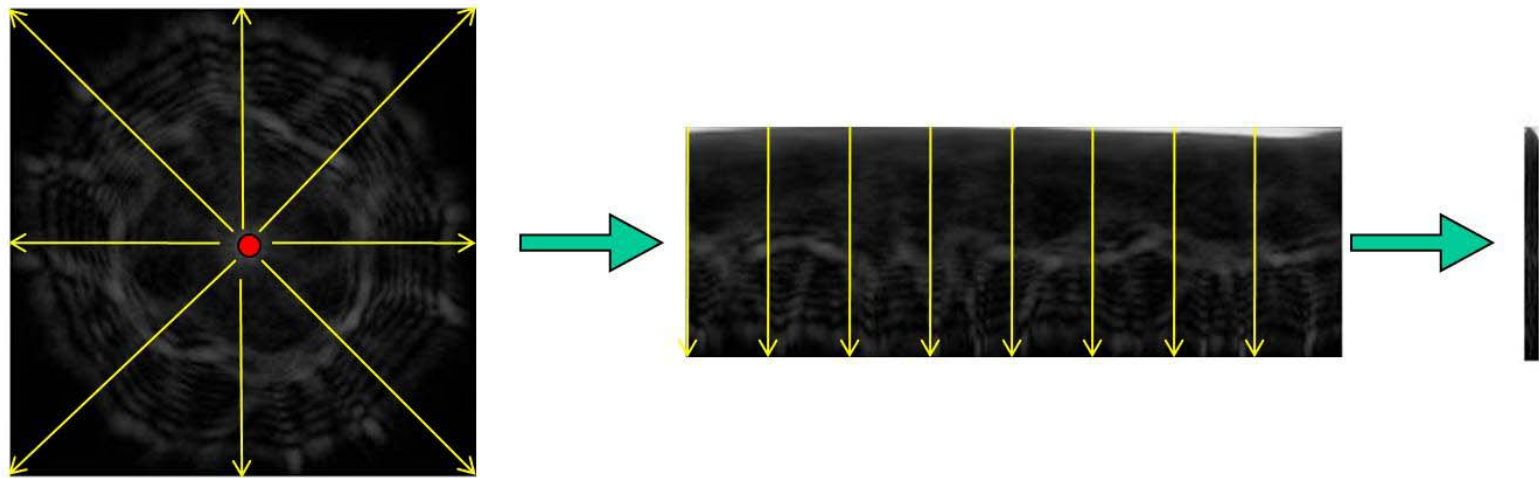


Feature Extraction



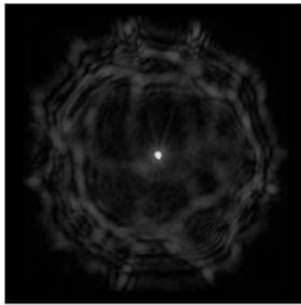
Resampled Image and three Levels
of Wavelet Components

Feature Extraction

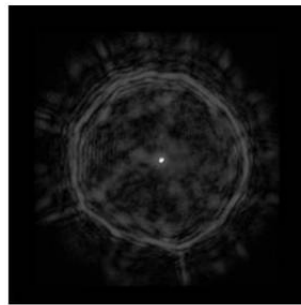


Gabor wavelets decomposition along 8 directions.

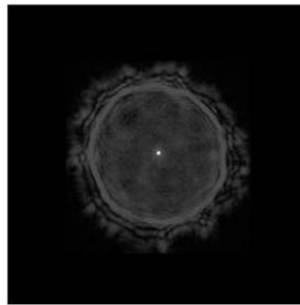
Feature Vectors



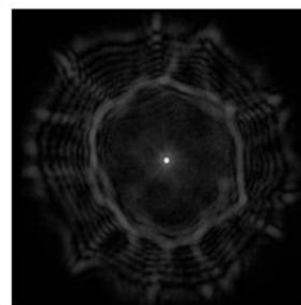
Cow B6



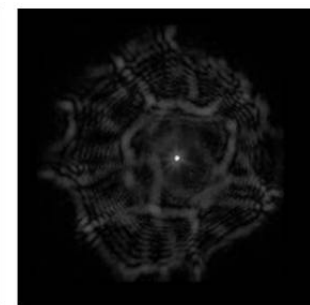
Goose B9



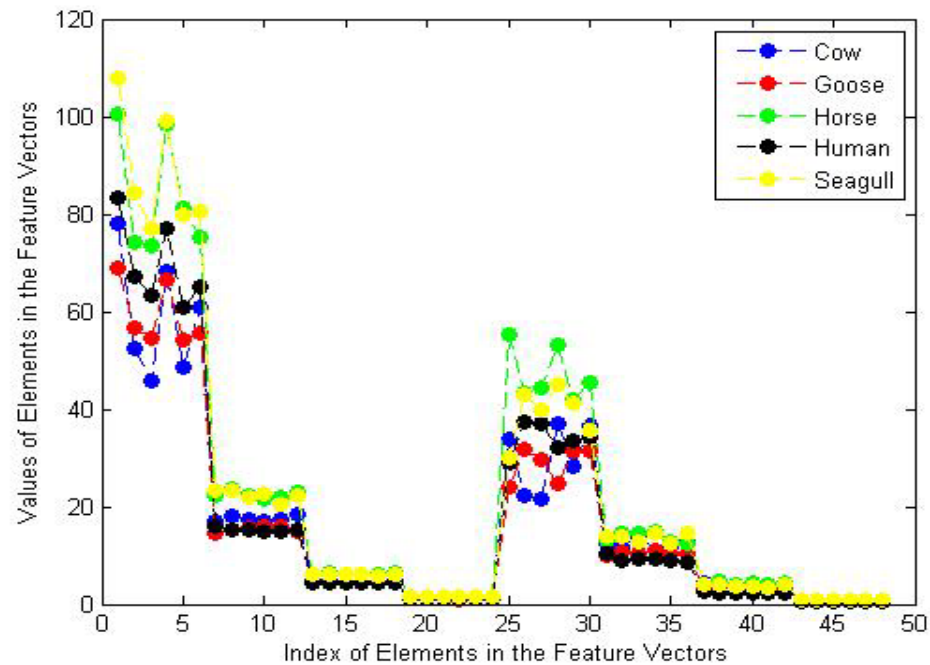
Horse E4



Human B4



Seagull 100



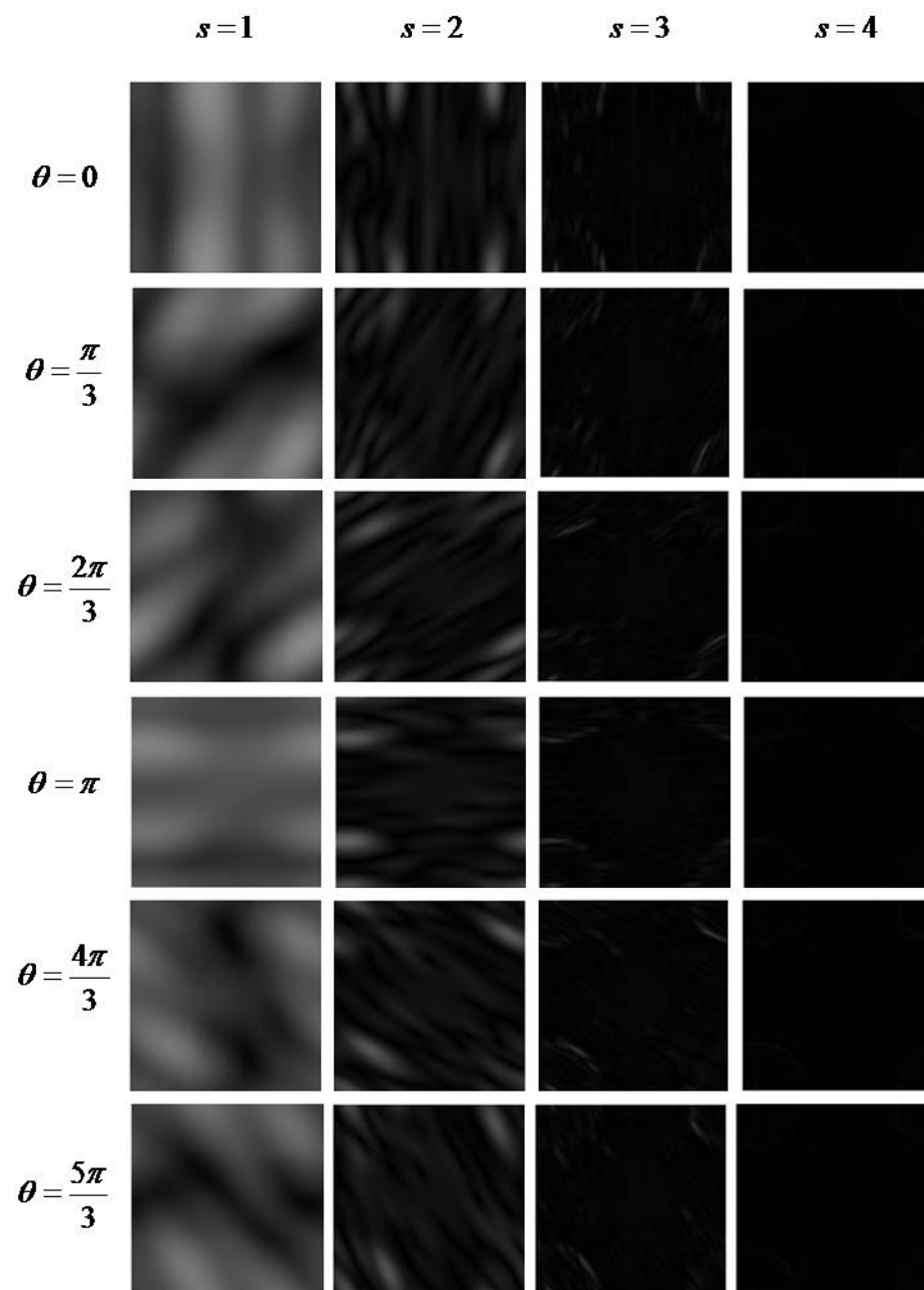
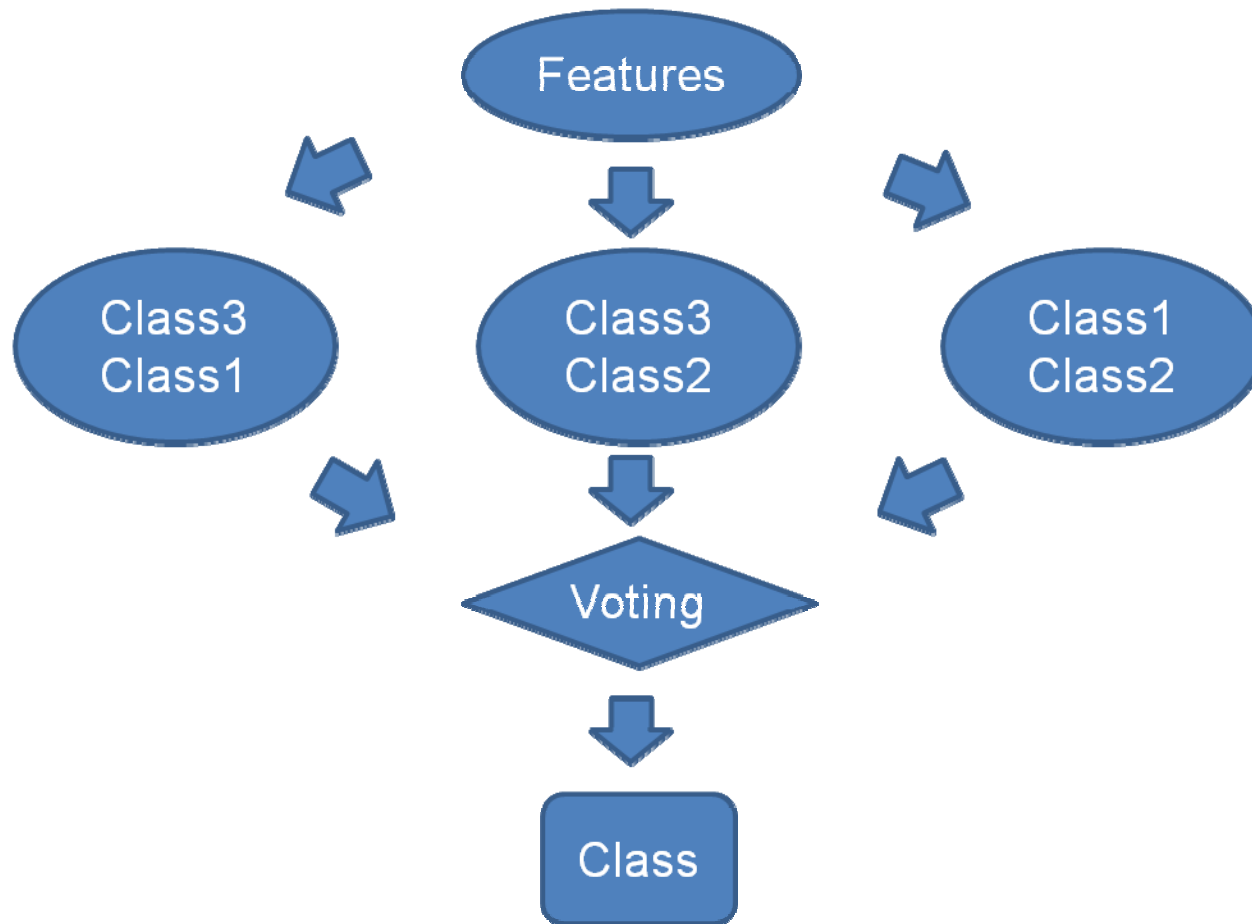
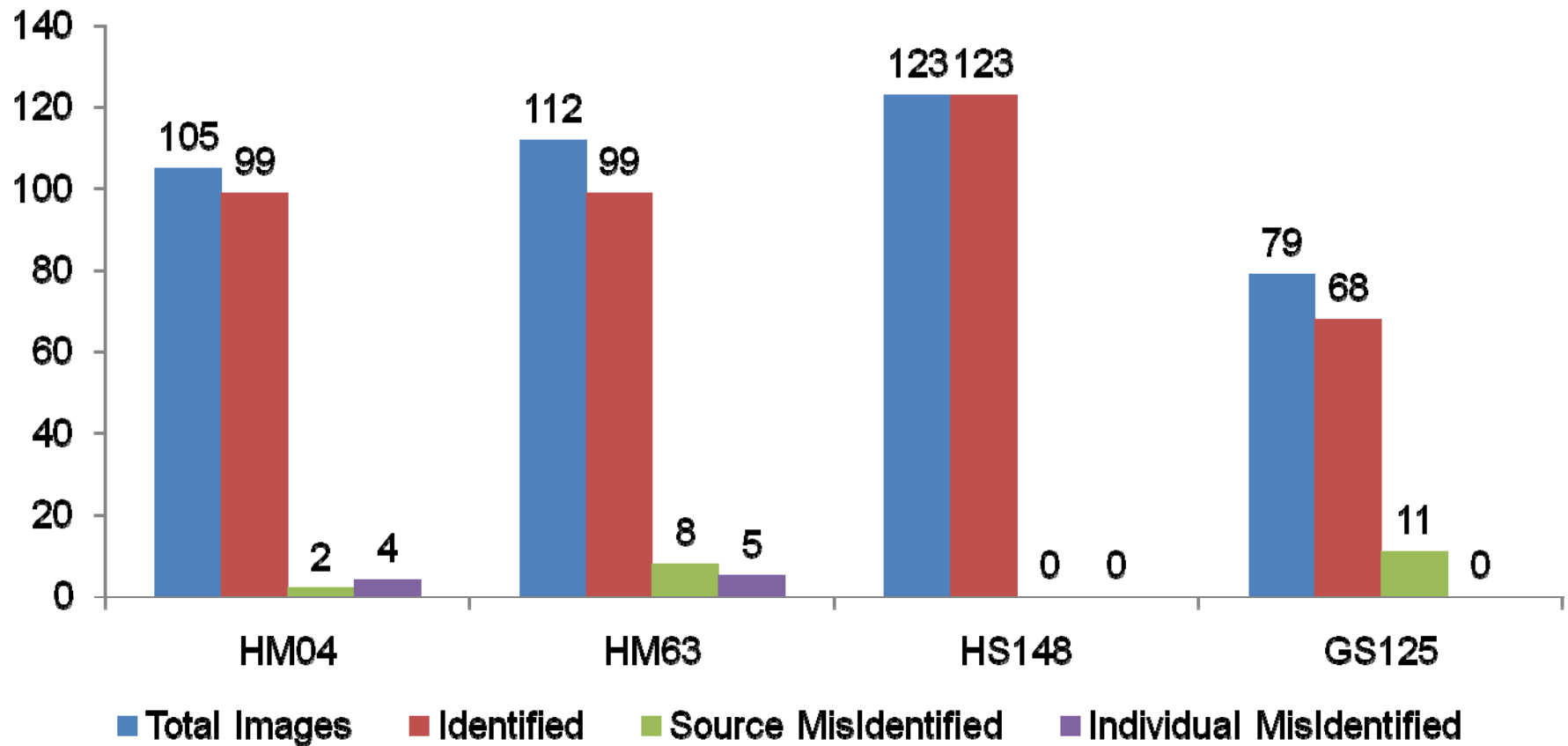


Image Classification



One-v-One Support Vector Machine (1-v-1 SVM)

Identification Results

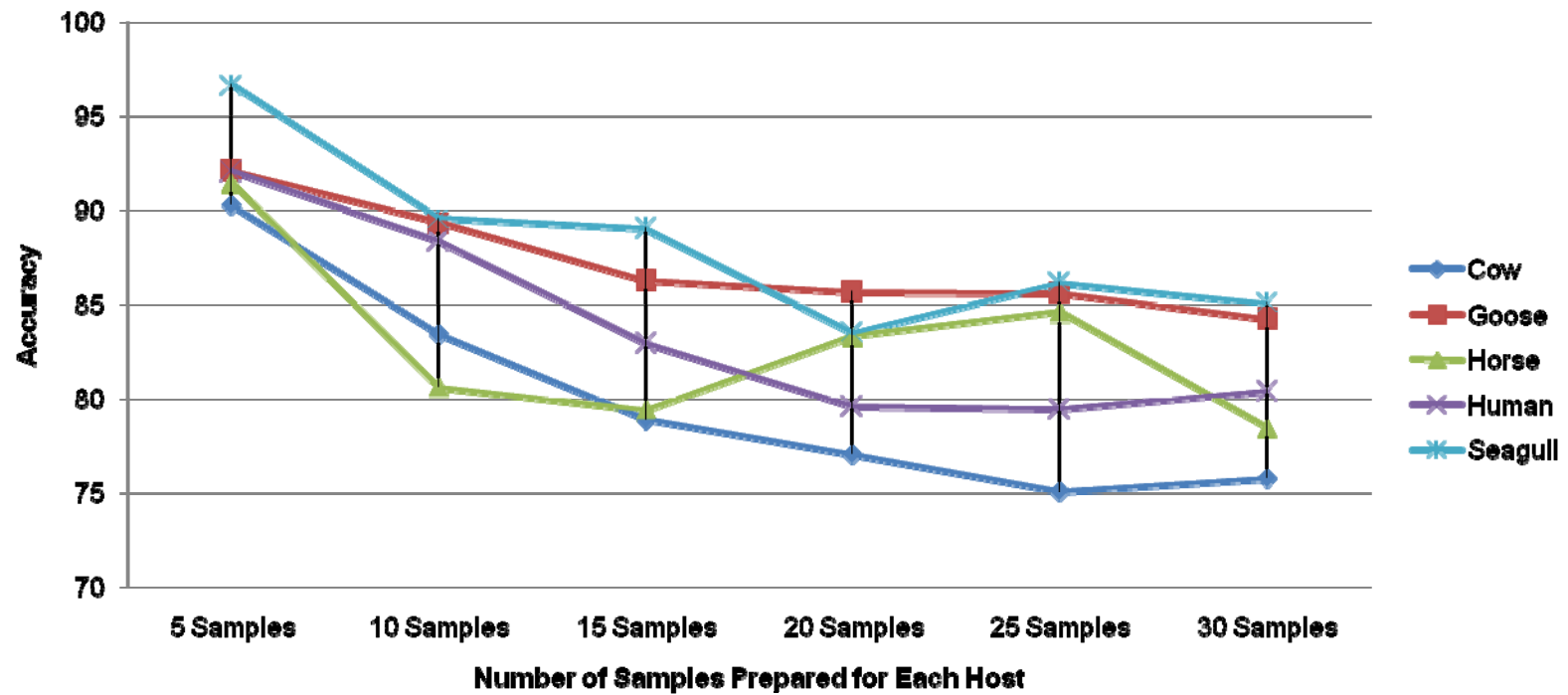


Accuracy: 93% Identified, 95% Source Identified.

Identification Results

| Host Species | Colonies | Identified | Accuracy |
|---------------------|-----------------|-------------------|-----------------|
| Cow | 308 | 255 | 82.8% |
| Goose | 301 | 267 | 88.7% |
| Horse | 299 | 246 | 82.3% |
| Human | 301 | 266 | 88.4% |
| Seagull | 297 | 266 | 89.6% |

Identification Accuracy



Conclusions

- Laser scatter imaging for MST
- High accuracy
- Easy to operate
- Automatic processing
- Remote or online MST using databases

Thank You