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Metrology of 3D Nanostructures

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Metrology of 3D Nanostructures

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Abstract

We propose a superresolution technique to resolve dense clusters of blinking emitters. The method relies on two basic assumptions: the emitters are statistically independent, and a model of the imaging system is known. We numerically analyze the performance limits of the method as a function of the emitter density and the noise level. Numerical simulations show that five closely packed emitters can be resolved and localized to a precision of 17nm. The experimental resolution of five quantum dots located within a diffraction limited spot confirms the applicability of this approach.

ACKNOWLEDGMENTS

The work reported here was performed at the University of Colorado at Boulder with funding provided through the LDRD office's Campus Executives program. The material in this SAND report is taken from a recent presentation of this research at the Optical Society of America's Computational Optical Sensing and Imaging topical conference (June 2012):

A. Barsic and R. Piestun, "Superresolution of Dense Quantum Dot Clusters using Independent Component Classification," in Computational Optical Sensing and Imaging, OSA Technical Digest (online) (Optical Society of America, 2012), paper CW2C.1.

Following the text of the manuscript, the slides presented at the meeting are shown in the appendix.

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1. INTRODUCTION

Optical microscopy has a fundamental resolution limit determined by diffraction. However, if one can assume the object consists of a collection of point-like sources, as is the case in single-molecule fluorescence imaging, the resolution paradigm is completely changed. Recent superresolution fluorescence microscopy techniques (such as PALM and STORM) rely on the ability to temporally resolve closely-spaced emitters to achieve superresolution [1,2]. The critical step is to control the experimental conditions such that unresolved emitters are switched temporally – in essence, temporal resolution is traded for spatial resolution. These methods still rely on the ability to resolve the emitters in time so that the images of different emitters do not overlap; therefore placing a limit on the labeling density of samples.

A related technique uses a clever analysis of quantum dot blinking; by exploiting their random temporal fluctuations, two emitters spaced closer than the diffraction limit can be resolved without relying on localization [3]. This method involves performing Independent Component Analysis (ICA) on the data. The way in which ICA is able to resolve the emitters relies on the fact that the quantum dots blink randomly and independently. The ICA algorithm decomposes the data into a small set of variables which have maximally non-Gaussian probability distribution functions. In principle, these non-Gaussian variables will correspond to the images of the independently blinking quantum dots in the scene.

Quantum dot emitters are attractive in various types of superresolution experiments due to their high photon output, photostability, wide range of emission wavelengths, and broad absorption spectra. Unfortunately, the ICA algorithm has one major shortcoming – in order to correctly resolve the emitters, one must know the true number of emitters in the scene (which is obviously not known in an experimental setting). This is a difficult enough hurdle to preclude the method from being implemented in any nontrivial situation. Here, we demonstrate a procedure that addresses this problem by incorporating spatial analysis of the quantum dots in addition to the stochastic temporal analysis. The algorithm uses ICA to generate a family of possible solutions and then the independent components are classified according to their spatial and temporal characteristics. The result that best matches a model of quantum dot behavior is selected – hence the name Independent Component Classification (ICC).

In this summary, we present numerical simulations to validate the superresolution capabilities of ICC. Subsequently, we apply the method to an experimental dataset in which five classically unresolvable quantum dots are successfully resolved.

2. INDEPENDENT COMPONENT CLASSIFICATION – NUMERICAL SIMULATIONS

In Monte Carlo simulations, several emitters were placed randomly within a diffraction limited spot, given random blinking behavior, and combined with background noise and shot noise. Then, the ICA was performed numerous times to generate a family of potential solutions. With each iteration, an increasing number of emitters was assumed. An example of such a solution set is shown in Figure 1. In this case, the true number of emitters is four. By examining the spatial characteristics of the potential solutions, a clear pattern emerges: once the number of emitters is overestimated, spurious results that do not resemble the system Point Spread Function (PSF) are returned. When the number of emitters is underestimated, the returned independent components are often a superposition of two emitters (this result is less obvious in this example where the emitters are unresolved, but simulations with easily resolved emitters clearly exhibited this tendency). ICC exploits this tendency in order to estimate the number of emitters in the scene. Each set of potential solutions is given a score that measures the maximum error of any one of the returned components as compared to the theoretical PSF. The number of emitters in the scene is assumed to be the set of results that immediately precedes the largest first derivative of the score set. In other words, the correct solution is assumed to be the one in which no spurious components are returned. This comparison is calculated by computing the L-2 norm of the difference between the data and the ideal PSF in Fourier space; the calculation is performed in Fourier space to account for possible lateral shifts.

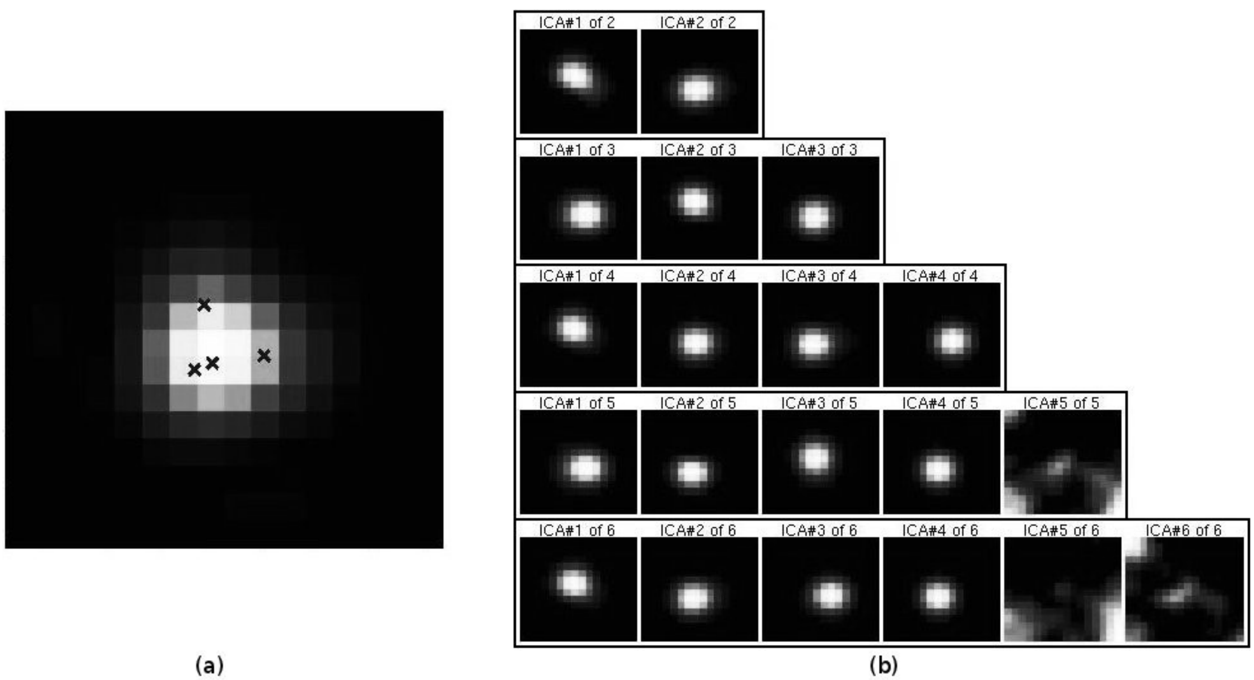


Figure 1. Simulation of Independent Component Classification: The average of the simulated video is shown in (a), with the centers of the unresolved quantum dots marked with x's. In (b), the family of potential solutions is shown. Each row is a separate result from the ICA algorithm, assuming a different number of emitters (row 1 assumes two emitters, row 2 assumes three emitters, etc.).

The results of the Monte Carlo simulations are summarized in Figure 2. For each number of emitters from one to seven, a random set of unresolved locations is generated, and the ICC algorithm attempts to determine the number of emitters and their locations; this is repeated 500 times for each number of emitters. As expected, the ability to correctly estimate the number of emitters increases with the SNR. With experimentally achievable SNRs, the ICC algorithm can reliably resolve up to five emitters within a diffraction limited spot. At higher densities, the number of emitters is often underestimated.

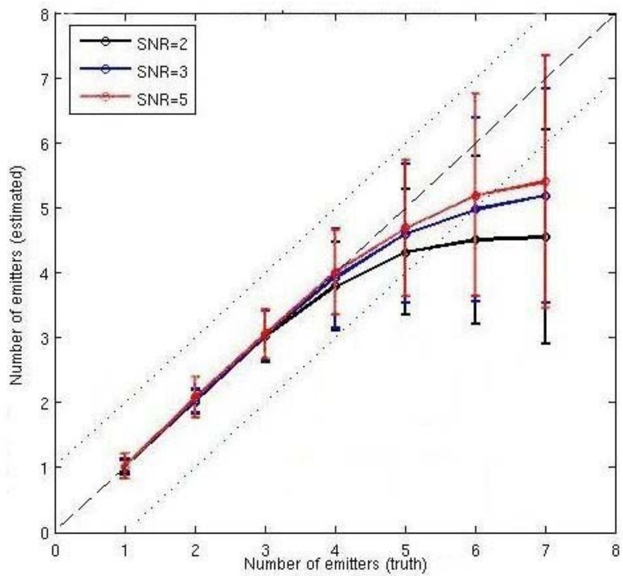


Figure 2. Results of Monte Carlo simulations for varying numbers of emitters and noise levels. Error bars show the standard deviation of the repeated simulations.

3. EXPERIMENTAL SUPERRESOLUTION RESULTS

Experiments were performed to test the ICC superresolution method. A fluorescence microscope was built using a 405nm diode laser as the excitation source, and a 1.3NA 100x Zeiss objective collected the fluorescence from the 525nm quantum dots from Invitrogen. A 100mm tube lens was selected to give 62.5x magnification on a Hamamatsu Orca-Flash 2.0 CMOS camera. This system resulted in slight over-sampling of the PSF, with 3.5 pixels across the full-width at half-maximum of the PSF. A test sample composed of quantum dots scattered across the cover slip was imaged for 500 frames with an exposure time of 200ms per frame, giving a total acquisition time of less than 2 minutes.

The results of the ICC analysis of a short video section are shown below. In the normal fluorescence image, there are no clearly-resolved emitters, but the ICC analysis suggests there are 5 unresolved emitters. Figure 3 shows the average image of the video, as well as the estimated locations of the emitters and their separated images. The distance between neighboring emitters is between 85 and 230nm in all cases, and the furthest distance between two of the emitters is 420nm. For comparison, this system's diffraction-limited spot is 493nm in diameter. Therefore, all 5 emitters have been superresolved.

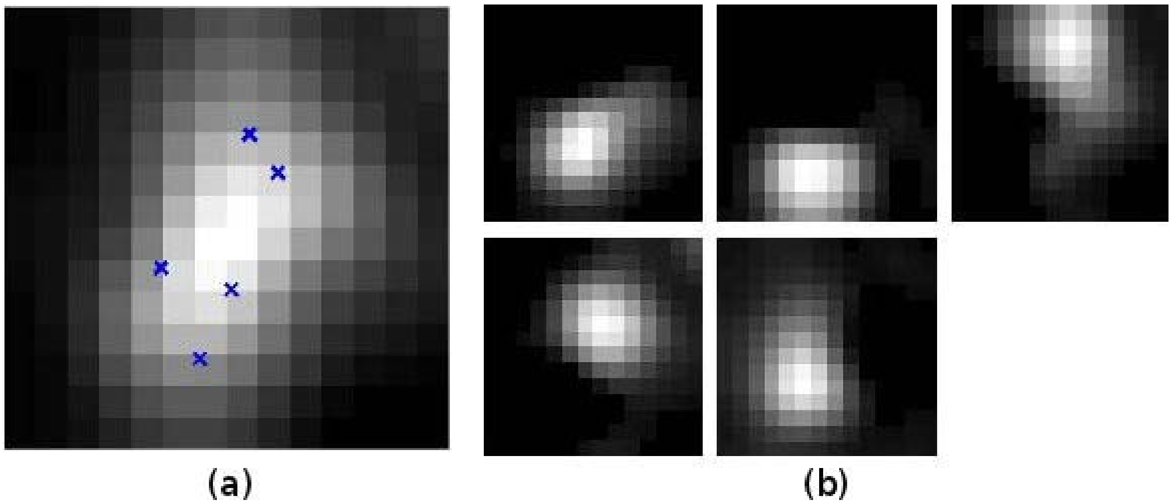


Figure 3. Experimental results: (a) shows the average of the 500-frame video of blinking quantum dots; the estimated locations of the five emitters are shown as x's. In (b), the five independent emitter images obtained with the ICC method are displayed.

4. SUMMARY

We demonstrated a superresolution technique capable of resolving dense clusters of quantum dots. The simulations validate the method, showing the ability to resolve emitters that would normally be unresolved. Experimental data shows superresolution well beyond the diffraction limit.

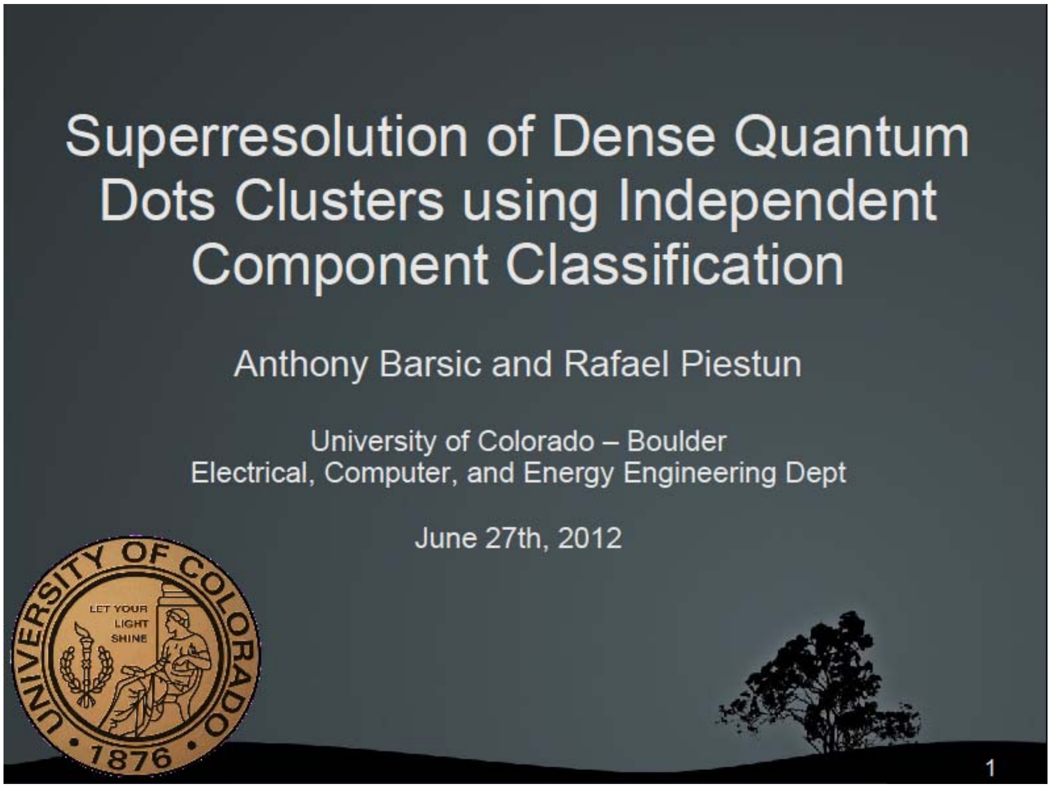
5. REFERENCES

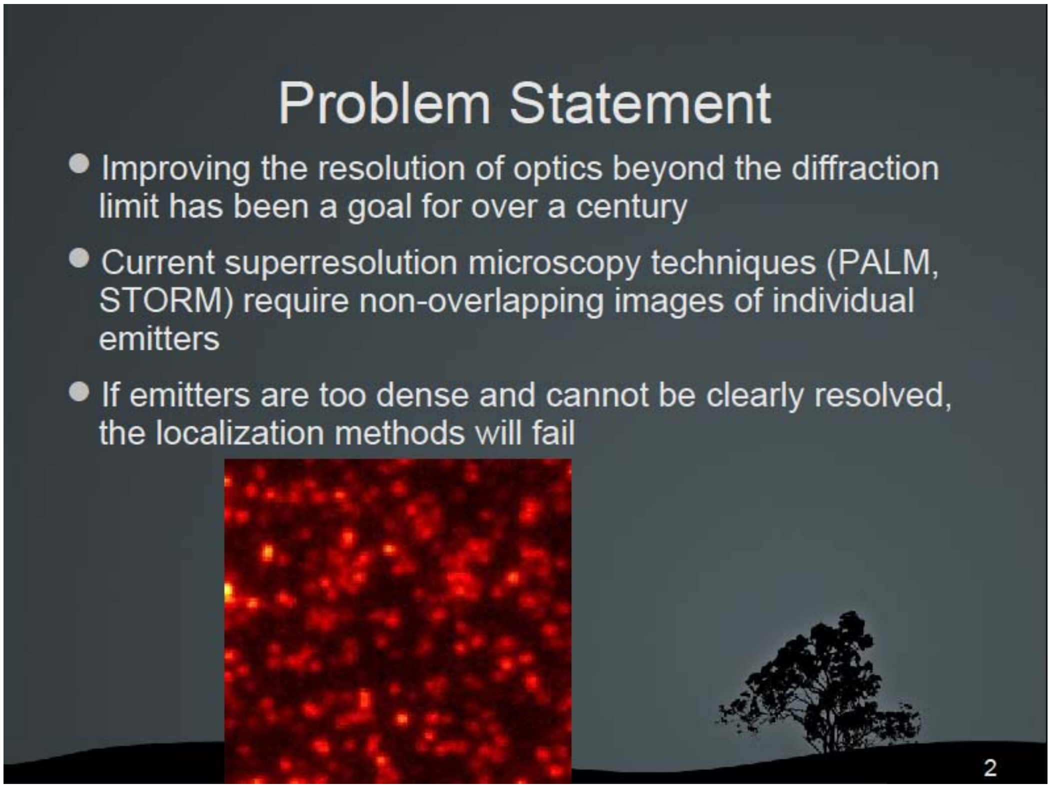
[1] E. Betzig, G. H. Patterson, R. Sougrat, O. W. Lindwasser, S. Olenych, J. S. Bonifacino, M. W. Davidson, J. Lippincott-Schwartz, and H. F. Hess, “Imaging intracellular fluorescent proteins at nanometer resolution,” Science 313, 1642–1645 (2006).

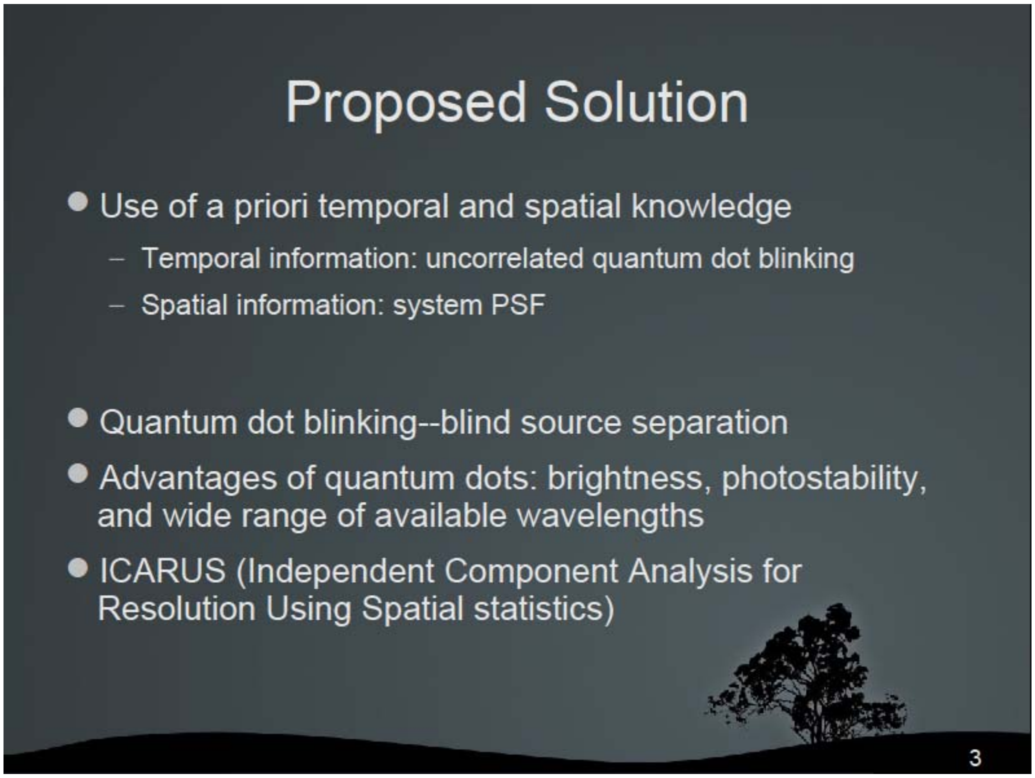
[2] M. J. Rust, M. Bates, and X. W. Zhuang, “Sub-diffraction-limit imaging by stochastic optical reconstruction microscopy (storm),” Nat. Methods 3, 793–795 (2006).

[3] K. A. Lidke, B. Rieger, T. M. Jovin, and R. Heintzmann, “Superresolution with quantum dots: enhanced localization in fluorescence microscopy by exploitation of quantum dot blinking,” Biophys. J. 88, 346a–346a (2005).

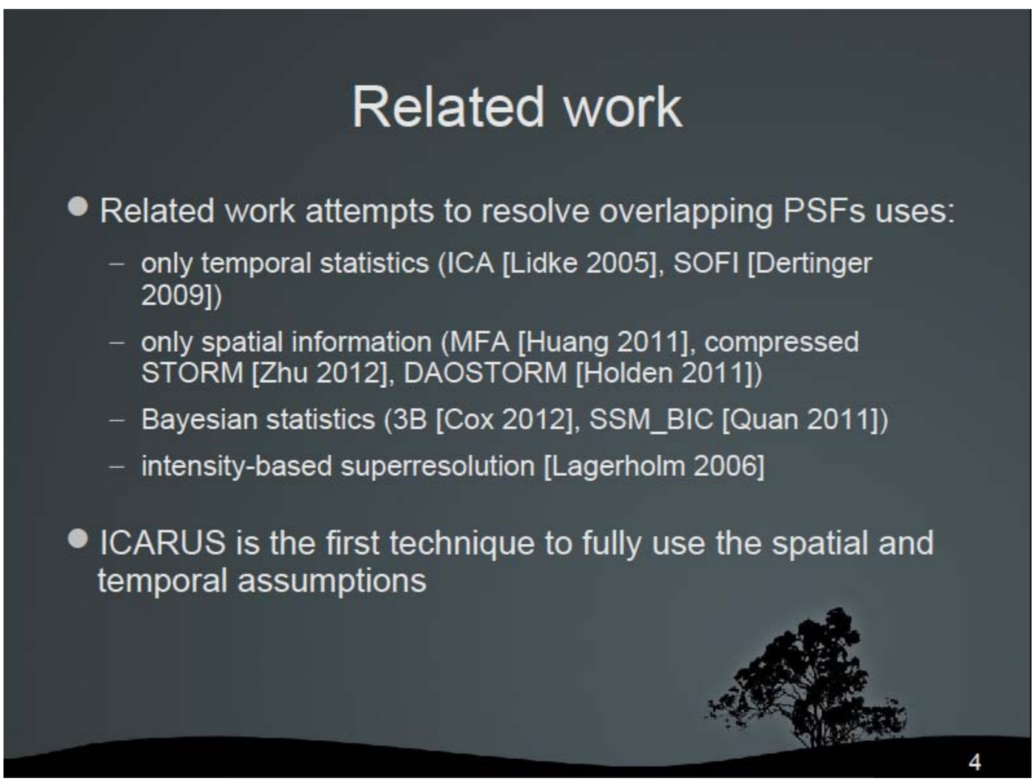
APPENDIX A: PRESENTATION SLIDES



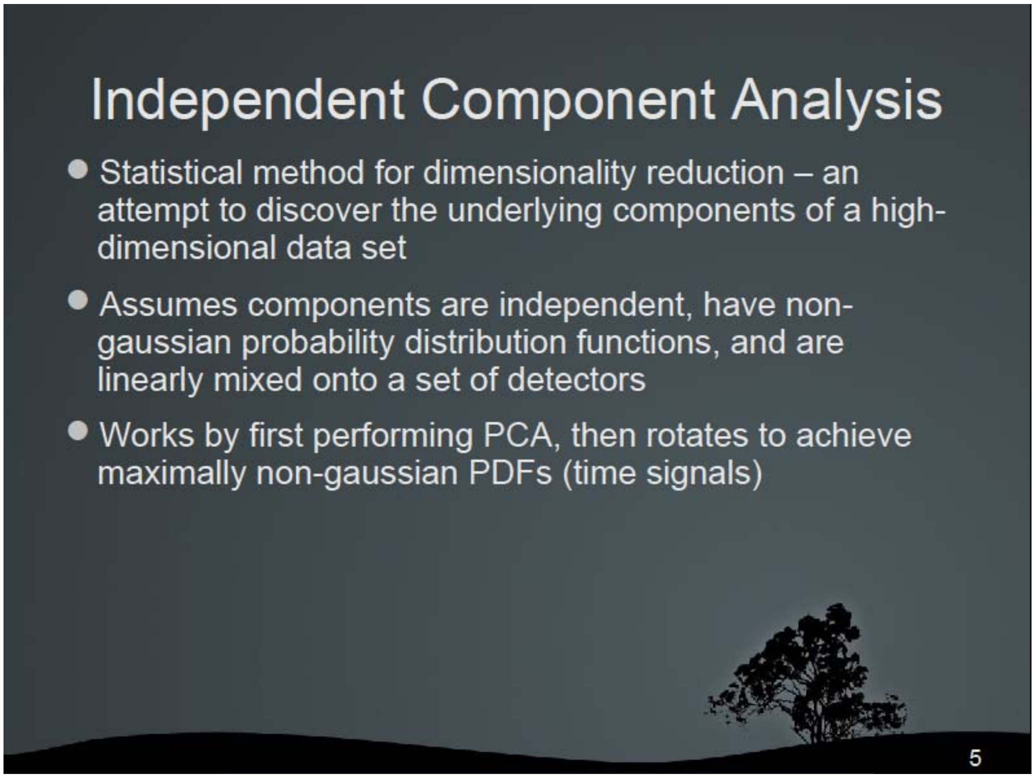


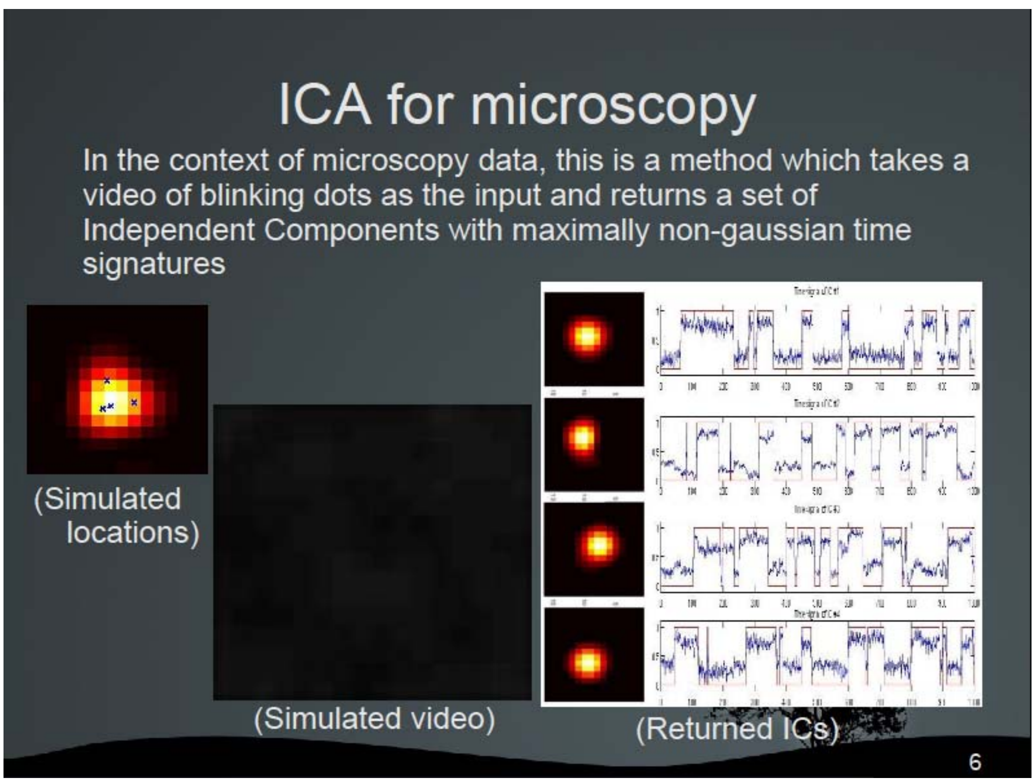


Proposed Solution
Use of a priori temporal and spatial knowledge
– Temporal information: uncorrelated quantum dot blinking
– Spatial information: system PSF
Quantum dot blinking--blind source separation
Advantages of quantum dots: brightness, photostability, and wide range of available wavelengths
ICARUS (Independent Component Analysis for Resolution Using Spatial statistics)
3



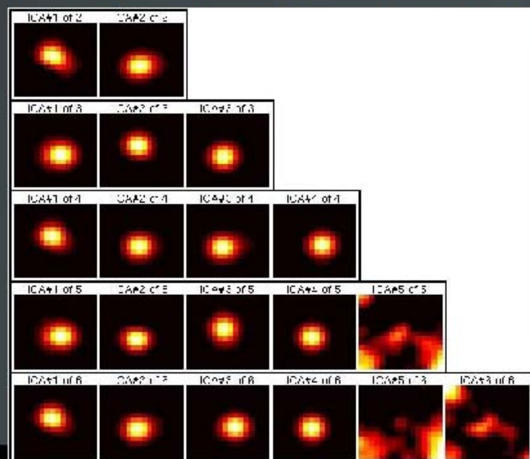
Related work
Related work attempts to resolve overlapping PSFs uses:
– only temporal statistics (ICA [Lidke 2005], SOFI [Dertinger 2009])
– only spatial information (MFA [Huang 2011], compressed STORM [Zhu 2012], DAOSTORM [Holden 2011])
– Bayesian statistics (3B [Cox 2012], SSM_BIC [Quan 2011])
– intensity-based superresolution [Lagerholm 2006]
ICARUS is the first technique to fully use the spatial and temporal assumptions
4


Independent Component Analysis
Statistical method for dimensionality reduction – an attempt to discover the underlying components of a high-dimensional data set
Assumes components are independent, have non-gaussian probability distribution functions, and are linearly mixed onto a set of detectors
Works by first performing PCA, then rotates to achieve maximally non-gaussian PDFs (time signals)
5


ICA for microscopy
In the context of microscopy data, this is a method which takes a video of blinking dots as the input and returns a set of Independent Components with maximally non-gaussian time signatures
(Simulated locations)
(Simulated video)
(Returned ICs)
6

Pyramid of Results (simulation)

- True number of emitters is not known
- Run ICA multiple times for different numbers of independent components to generate a set of potential solutions



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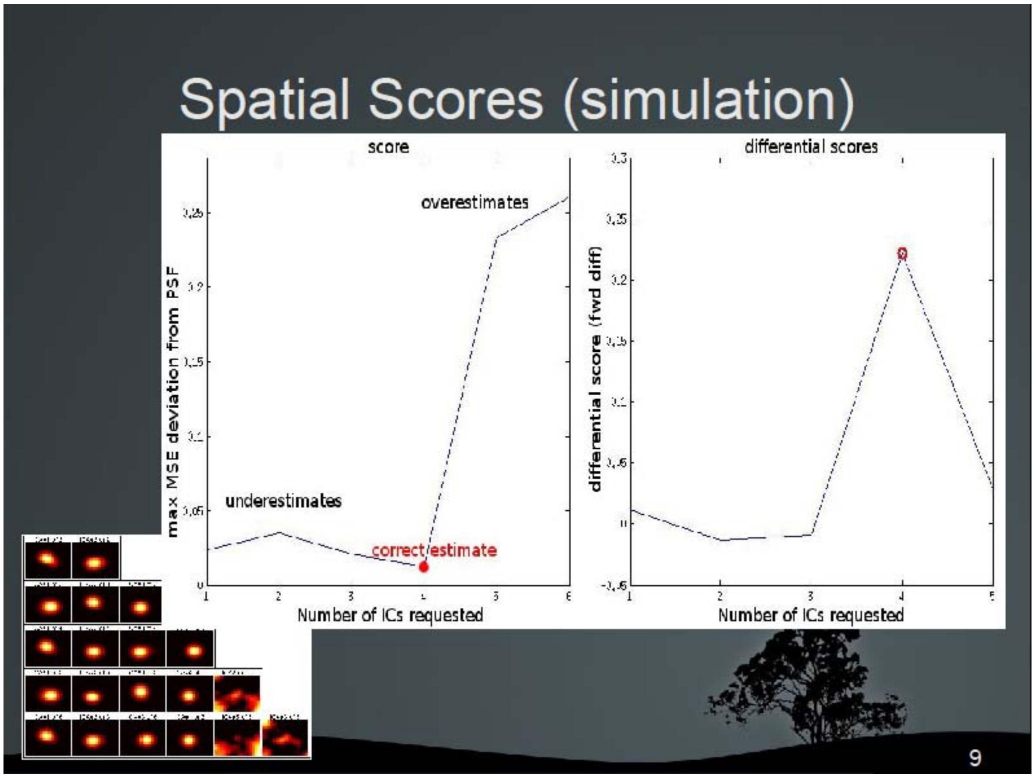
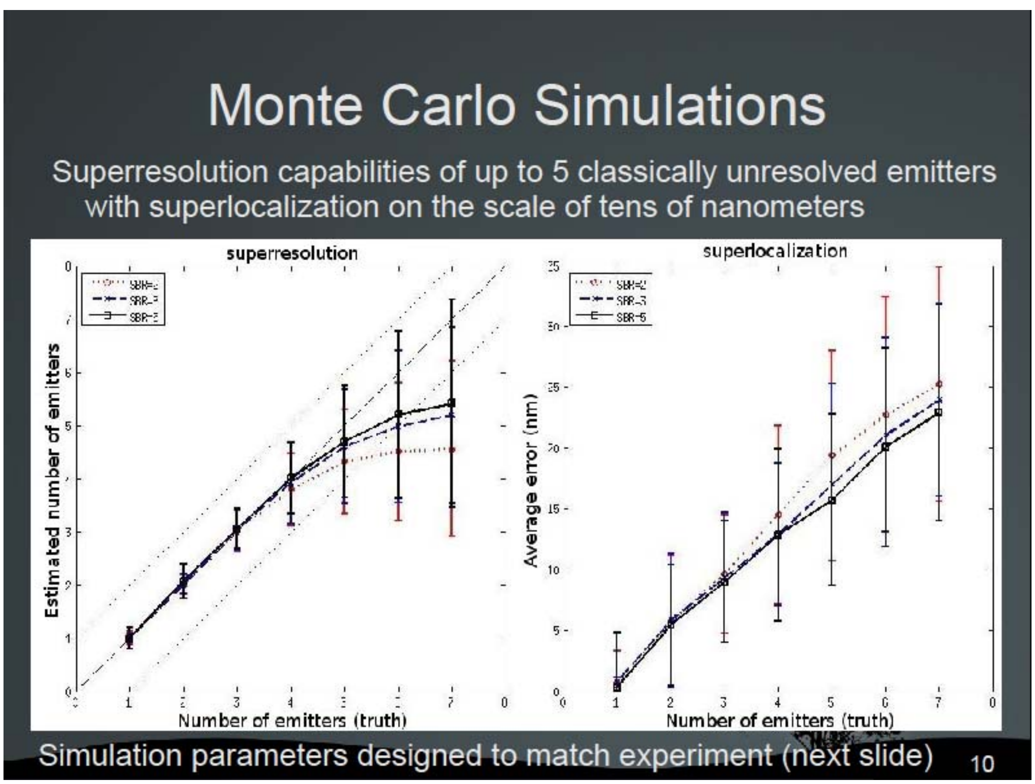
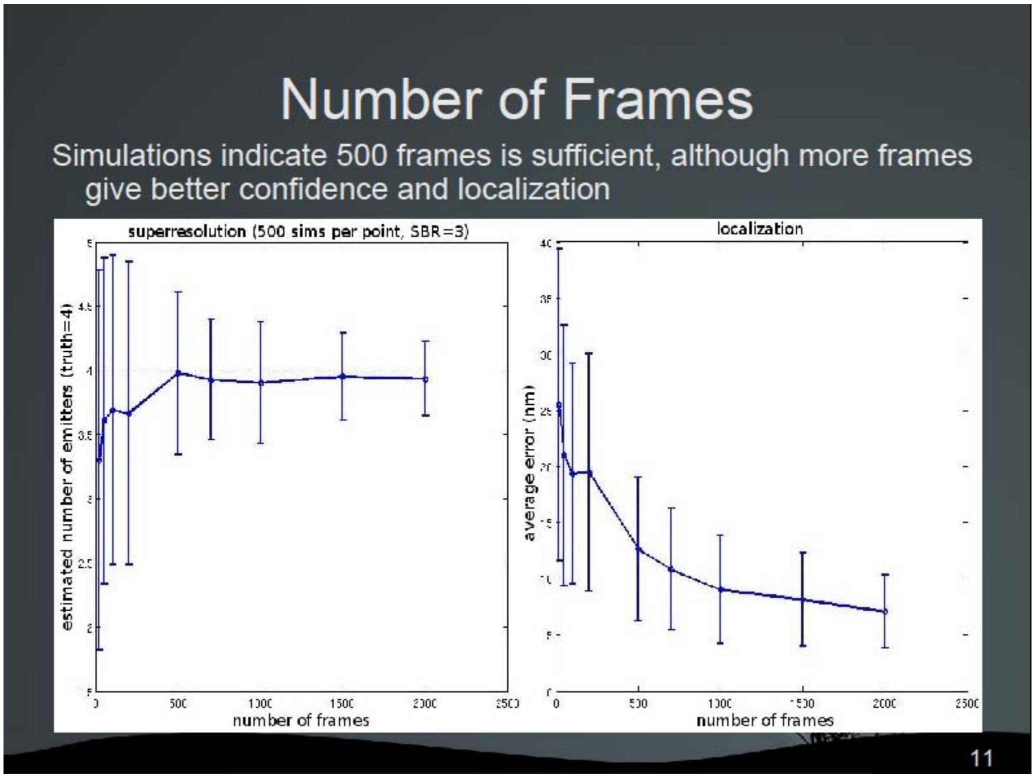
Spatial Scoring - ICARUS

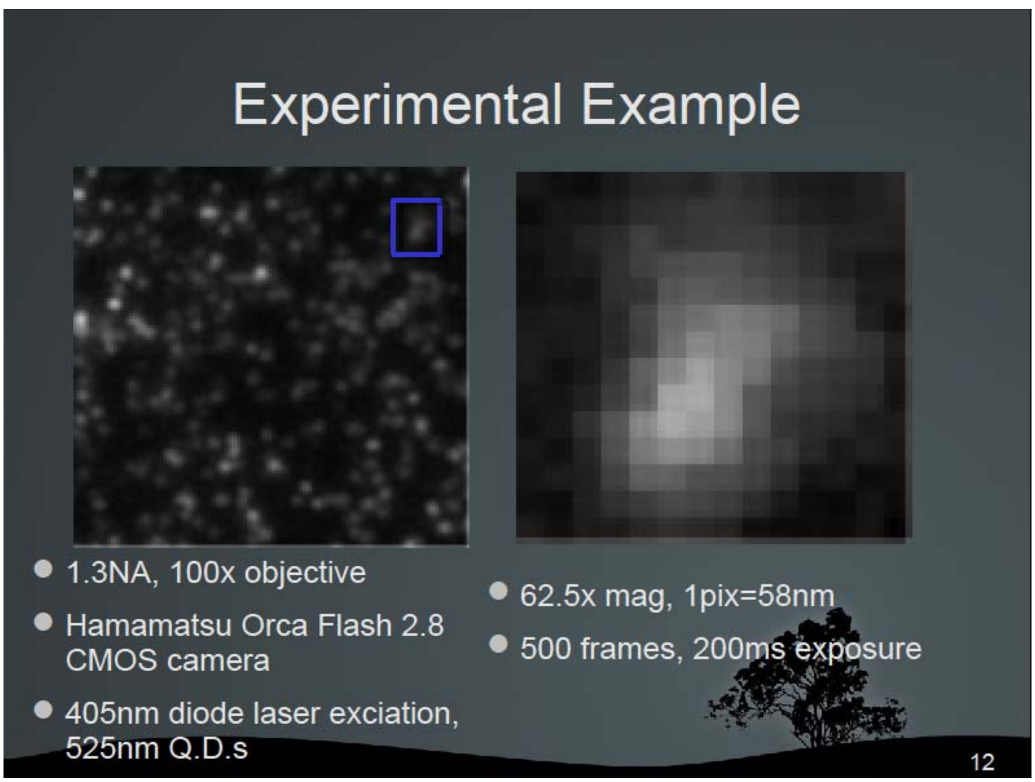
- Fourier scoring – do 2DFFT of each IC and compare with 2DFFT of ideal PSF
- Score = $\sum || F\{IC\} - F\{PSF\}||_2^2$
- For each solution set (row), pick the worst (highest) score as the indicator of success/failure
- Hence the name: ICARUS (Independent Component Analysis for Resolution Using Spatial statistics)

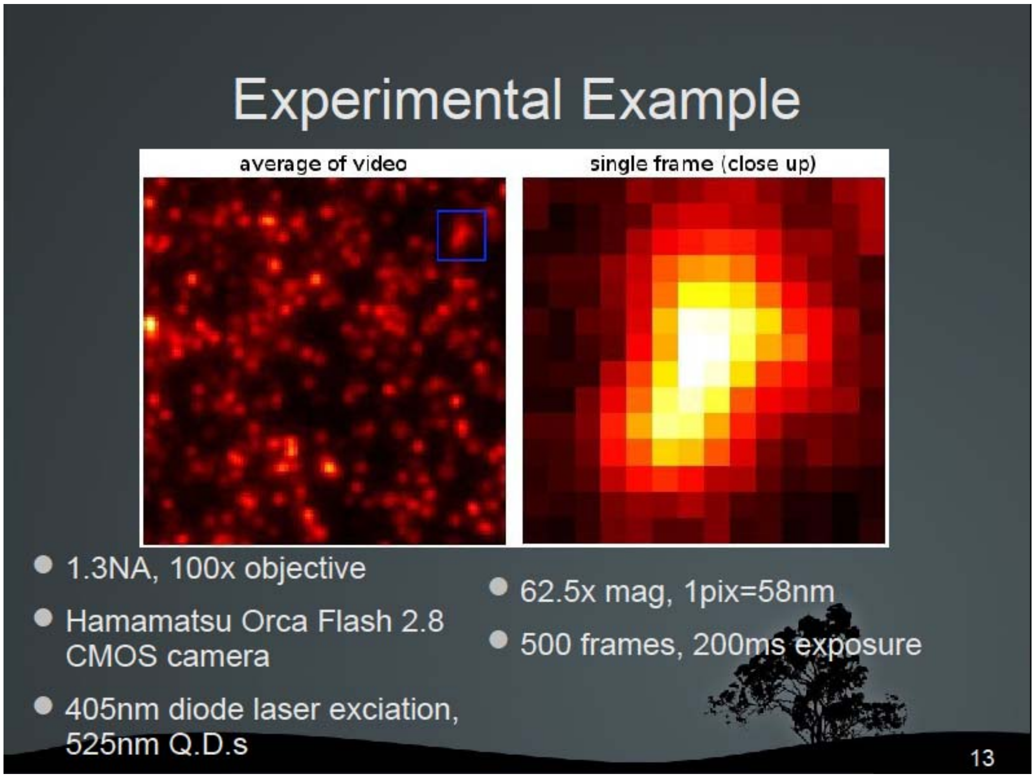
8

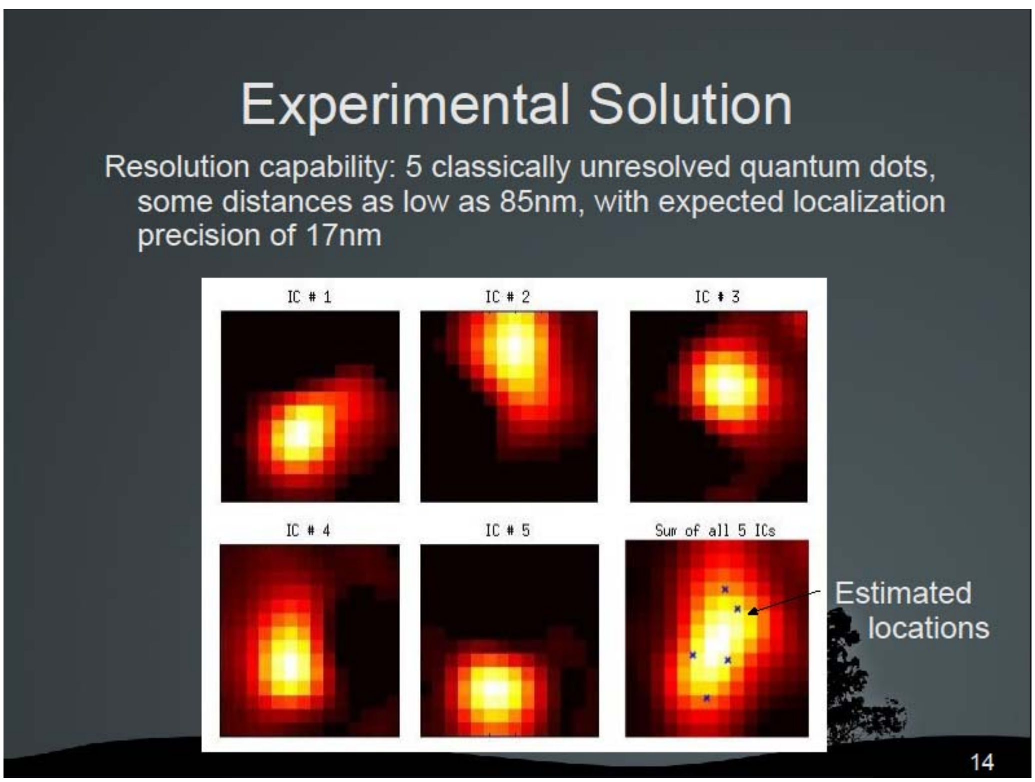

Spatial Scores (simulation)
score
differential scores
overestimates
underestimates
correct estimate
max MSE deviation from PSF
differential score (fwd diff)
Number of ICs requested
Number of ICs requested
9


Monte Carlo Simulations
Superresolution capabilities of up to 5 classically unresolved emitters with superlocalization on the scale of tens of nanometers
superresolution
superlocalization
Estimated number of emitters
Average error (nm)
Number of emitters (truth)
Number of emitters (truth)
Simulation parameters designed to match experiment (next slide)
10


Number of Frames
Simulations indicate 500 frames is sufficient, although more frames give better confidence and localization
superresolution (500 sims per point, SBR=3)
estimated number of emitters (truth=4)
number of frames
localization
average error (nm)
number of frames
11


Experimental Example
1.3NA, 100x objective
Hamamatsu Orca Flash 2.8 CMOS camera
405nm diode laser exciation, 525nm Q.D.s
62.5x mag, 1pix=58nm
500 frames, 200ms exposure
12


Experimental Example
average of video
single frame (close up)
1.3NA, 100x objective
Hamamatsu Orca Flash 2.8 CMOS camera
405nm diode laser exciation, 525nm Q.D.s
62.5x mag, 1pix=58nm
500 frames, 200ms exposure
13


Experimental Solution
Resolution capability: 5 classically unresolved quantum dots, some distances as low as 85nm, with expected localization precision of 17nm
IC # 1
IC # 2
IC # 3
IC # 4
IC # 5
Sum of all 5 ICs
Estimated locations
14

Summary

- ICARUS is a superresolution method for dense clusters of blinking quantum dots
- Uses both spatial and temporal information
- Simulations indicate the ability to resolve up to 5 emitters located within the area of a diffraction-limited spot with 17nm precision
- Experiments confirm that ICARUS is realistically applicable in typical conditions

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Thanks

- NSF IGERT program in Computational Optical Sensing and Imaging
- Sandia National Labs: Engineering Excellence Fellowship
- The whole research group

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