Trade-offs to win-win
Krishnaswami, V.

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# List of acronyms

1. **ADC**: Analog to digital converter  
2. **AIM**: Active (or adaptive) illumination microscopy  
3. **AOTF**: Acousto-optic tunable filter  
4. **AOM**: Acoustic optical modulator  
5. **AU**: Airy unit  
6. **CCD**: Charge coupled device  
7. **CGF**: Conversion gain factor  
8. **CLEM**: Controlled light exposure microscopy  
9. **CLSM**: Confocal laser scanning microscopy  
10. **CRLB**: Cramer-rao lower bound  
11. **CS**: Compressed sensing  
12. **DMD**: Digital micromirror device  
13. **DSLM**: Digital Scanned Laser Light-Sheet Fluorescence Microscopy  
14. **EMCCD**: Electron multiplying charge coupled devices  
15. **ENF**: Excess noise factor  
16. **FA**: Feedback active  
17. **FF**: Fill factor  
18. **FLIM**: Fluorescence lifetime imaging measurement  
19. **FPN**: Fixed pattern noise  
20. **FPGAs**: Field programmable gate arrays  
21. **HDR**: High dynamic range  
22. **HyDs**: Hybrid detectors  
23. **ICs**: Integrated circuits  
24. **MLE-PAM**: Minimized light exposure programmable array microscope  
25. **NA**: Numerical Aperture
26. **PALM**: Photoactivated localization microscopy
27. **PAM**: Programmable array microscope
28. **PDE**: Photon detection efficiency
29. **PDP**: Photon detection probability
30. **PID**: Proportional integral derivative
31. **PL**: Power limited
32. **PSF**: Point spread function
33. **QE**: Quantum efficiency
34. **RCM**: Rescan confocal microscopy
35. **REScue STED**: Reduction of excitation and signal suppression cycles
36. **RF**: Redistribution factor
37. **ROS**: Reactive oxygen species
38. **SCIM**: Spatially-controlled illumination microscopy
39. **sCMOS**: Scientific complementary metal oxide semiconductor
40. **sGFP**: Superfolder green fluorescent protein
41. **SF**: SCIM factor
42. **SIM**: Structured illumination microscopy
43. **SLM**: Spatial light modulator
44. **SNR**: Signal-to-noise ratio
45. **SPAD**: Single-photon avalanche diode array
46. **SPIM**: Selective Plane Illumination Microscopy
47. **STED**: Stimulated emission depletion
48. **STORM**: Stochastic optical reconstruction microscopy
49. **TIRF**: Total internal reflection fluorescence
50. **TLS-SPIM**: Tiling Light-Sheet Selective Plane Illumination Microscopy
Summary

“Seeing is believing” is a popular idiom, coined in the 17th century. Microscopy enables the visualization of entities that are not visible to our naked eye. The world’s first microscopes were built in The Netherlands during the period between 16th-17th century, notably by Zacharias Janssen, Hans Lippershey, Jan Swammerdam, and Antonie van Leeuwenhoek. At that time, images were manually sketched, by microscopists to study the underlying structures which were visualized with a microscope. Today, microscopy has grown leaps and bounds with the development of optics, sensors and computer technology.

There are two main branches in microscopy, namely electron microscopy and optical microscopy. In optical microscopy, light is used for imaging. It can be combined with fluorescence imaging, where fluorescent probes are tagged onto specific structures of biological specimens. Consequently, imaging of living and fixed biological specimens can be performed with a high degree of specificity. Imaging is performed by illuminating the specimen to cause excitation of fluorescent molecules that leads to the generation of fluorescence emission signal. This signal is captured by a sensitive camera or detector to obtain an image that represents the fluorescence distribution in the specimen. The image quality is determined by the amounts of signal captured during imaging. Therefore, greater amounts of fluorescence excitations leads to improved image quality. The flipside of greater fluorescence excitations is the occurrence of photodamage, which includes both phototoxicity and photobleaching. Photodamage occurs when toxic reactive oxygen species are created.
during imaging, reacts with the fluorescent specimen leading to loss in fluorescence signal and/or death of the specimen. This leads to a trade-off between image quality and photodamage. This can be addressed by improving imaging sensitivity which enables the extraction of the spatial information of the specimen with optimal number of fluorescence excitations during imaging.

Conventional fluorescence imaging methodology involves the application of spatially-uniform illumination with a fixed intensity level. Therefore, number of unnecessary fluorescence excitations can occur during imaging. For example, fluorescence excitations that occur outside the focus plane for imaging can be deemed unnecessary. Such excitations do not contribute to image quality but can lead to the occurrence of photodamage. With little control of the illumination process, the overall amounts of fluorescence excitations can either be increased or decreased by changing the fixed intensity level used for illumination. Therefore, a more ‘smart-imaging’ paradigm can be useful for fluorescence imaging.

In this thesis, chapter 2 reviews the concepts of ‘spatially-controlled illumination microscopy’ (SCIM) which adopts the application of spatially-variable illumination with multiple or fixed intensity levels for imaging. Using this technique, the overall illumination levels and hence the fluorescence excitations can be reduced to limit photodamage without compromising the image quality.

Chapter 3 and 4 of this thesis focuses the implementation of SCIM in rescan confocal microscopy (RCM) for improving imaging sensitivity. RCM
is an improved confocal microscopic technique, that is capable of achieving optimal lateral resolution with an open pinhole configuration thereby enabling greater amounts of signal detection. Therefore, imaging can be performed with reduced illumination levels. In combination with SCIM, the illumination levels and hence the fluorescence excitations can be further reduced, without any compromise in the image quality. Hence, with constructive combination of SCIM and RCM imaging sensitivity can be improved.

Chapter 5 of this thesis demonstrates the applicability of SCIM imaging to not only limit photodamage, but also improve the image quality. Here, illumination levels are spatially-increased or spatially-decreased at different regions of the fluorescent specimen. The extent upto which the illumination levels are increased or decreased can be controlled by the user. It is shown that with spatial-control of illumination optimal amounts of fluorescence excitations can be achieved to both improve image quality and limit photodamage at the same time.

Chapter 6 of this thesis focuses on scope for improvements in detector/sensor technology for improving imaging sensitivity. The use of digital photon counting detectors, with single-photon sensitivity and zero read-out noise, its application for fluorescence microscopy, particularly in the context of super-resolution imaging is discussed.

Finally, in chapter 7, a discussion of SCIM technology, its advantages, limitations and future prospects are outlined.
Samenvatting

"Seeing is believing" is een populaire idioom, gecreëerd in de 17e eeuw. Microscopie maakt visualisatie mogelijk van entiteiten die niet zichtbaar zijn voor ons blote oog. De eerste microscopen ter wereld werden in de 16-17e eeuw gebouwd in Nederland, voornamelijk door Zacharias Janssen, Hans Lippershey, Jan Swammerdam, and Antonie van Leeuwenhoek. Op dat moment werden beelden nog vaak handmatig geschetst door microscopisten om de onderliggende structuren te bestuderen die de microscoop visualiseerde. Tegenwoordig is microscopie sterk geëvolueerd aan de hand van de ontwikkeling van optica, sensoren en computertechnologie.

Er zijn twee hoofdtakken in microscopie, namelijk elektronenmicroscopie en optische microscopie. Bij optische microscopie wordt licht gebruikt voor beeldvorming. Het kan gecombineerd worden met fluorescentie beeldvorming, waar fluorescerende labels specifieke structuren van biologische samples aankleuren. Hierdoor kan beeldvorming van levende en gefixeerde biologische samples met een hoge mate van specificiteit worden uitgevoerd. Beeldvorming wordt uitgevoerd door het sample te belichten waardoor excitatie van de fluorescerende moleculen wordt veroorzaakt die leiden tot een fluorescent emissiesignaal. Dit signaal wordt gemeten door een gevoelige camera of detector voor het verkrijgen van een afbeelding die de fluorescentieverdeling in het monster vertegenwoordigt. De beeldkwaliteit wordt bepaald door de hoeveelheid signaal dat wordt opgevangen. Daarom leidt een grotere hoeveelheid fluorescentie excitaties tot verbeterde beeldkwaliteit. De keerzijde van
veel fluorescentie-excitaties is het optreden van fotoschade, die zowel fototoxiciteit als fotobleking omvat. Fotoschade treedt op wanneer toxische zuurstofradicalen worden gevormd. Deze reageren met het fluorescerende sample en leidt tot verlies in fluorescentiesignaal en/of dood van het levende biologische sample. Dit leidt tot een afweging tussen beeldkwaliteit en fotoschade. Dit kan worden aangepakt door een nieuwe methode die gebruikt maakt van de ruimtelijke verdeling van de fluorescentie, waardoor een optimaal aantal fluorescentie-excitaties tijdens beeldvorming mogelijk is.


In dit proefschrift beschouwt hoofdstuk 2 de concepten van 'ruimtelijk gecontroleerde belichtingsmicroscopie' (SCIM) over de toepassing van ruimtelijk-variabele belichting met meerdere intensiteitsniveaus in beeldvorming. Met behulp van deze techniek wordt het totale belichtingsniveau en dus de fluorescentie-excitaties worden verminderd.
waardoor fotoschade wordt beperkt zonder de beeldkwaliteit in gevaar te brengen.

Hoofdstuk 3 en 4 van dit proefschrift richten zich op de toepassen van SCIM in ‘Rescan’ Confocale Microscopie (RCM) om de beeldgevoeligheid nog verder te verbeteren. RCM is een verbeterde confocale microscopische techniek, die in staat is om een optimale laterale resolutie te bereiken met een open ‘pinhole’ configuratie waardoor een grotere hoeveelheid signaal detectie mogelijk is. Hierdoor kan beeldvorming worden uitgevoerd met verminderde belichtingsniveaus. In combinatie met SCIM kunnen de belichtingsniveaus en dus de fluorescentie-excitaties verder worden verminderd, zonder verlies in de beeldkwaliteit. Dus met een constructieve combinatie van SCIM en RCM beeldgevoeligheid kan worden verbeterd.

Hoofdstuk 5 van dit proefschrift toont aan dat SCIM imaging niet alleen toepasbaar is om fotoschade te beperken, maar ook om de beeldkwaliteit te verbeteren. Hier worden de belichtingsniveaus ruimtelijk verhoogd of ruimtelijk verlaagd in verschillende gebieden van het fluorescerende sample. De mate waarin de belichtingsniveaus worden verhoogd of verlaagd, kunnen door de gebruiker worden bepaald. Het blijkt dat bij ruimtelijke controle van belichting een optimale hoeveelheid fluorescentie-excitaties kan worden bereikt die tegelijkertijd de beeldkwaliteit verbeteren en fotoschade beperken.

Hoofdstuk 6 van dit proefschrift richt zich op mogelijkheden voor verbeteringen in detector/sensor technologie voor het verhogen van
beeldgevoeligheid. Het gebruik van een digitale fotonentellers, met een gevoeligheid van één foton en zonder ruis, wordt getest in fluorescentiemiicroscopie, in bijzonder in het kader van super-resolutiebeelden.

Ten slotte worden in hoofdstuk 7 een discussie over SCIM-technologie, de voordelen, beperkingen en toekomstperspectieven uiteengezet.
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List of publications


