Introduction to Optical Microscopy-Objectives

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In this session we will explore the technical aspects of the heart of the optical microscope-the objective lens. The evolution of the objective in the past decade has been exceptional. Driven by the demands of biological, biomedical, and material science researchers coupled with advances in lens computer design, lens coating technology, and glass formulation chemistry, this evolution continues First we will briefly review some historical background, followed by current terminology, and ending with objective oriented optical techniques.

Early nineteenth century microscopists were plagued by a lack of image quality including blurring and various chromatic, spherical and axis aberrations caused by design flaws and glass raw material quality. In the mid nineteenth century work by Lister and Amici reduced chromatic aberration and raised the numerical aperture by introducing the first achromatic objectives. In the last part of the nineteenth century Ernst Abbe and Carl Zeiss collaborated to produce the first apochromatic objectives. These apochromats again increased numerical aperture, reduced spherical aberration, and most importantly corrected for chromatic aberration almost perfectly [2].

In order to fully understand objectives some basic terms need to be discussed briefly. Numerical aperture (NA) simply defined, is the ability of an objective to resolve fine detail coupled with its ability to gather light expressed as a number [1]. The higher the NA of an objective the greater the resolution and brightness of the observed image. All dry objectives have NA's lower than 1 whereas oil objectives generally greater than 1. Objectives are made of various types of glass. The simplest type of objective, Achromat, is made of crown and flint glass elements. The next level of objective is called a Fluorite is composed of elements of calcium fluorite and is some cases quartz glass permitting transmission in the UV range (340nm). The most highly corrected objective is the Apochromat. It is composed of elements of fluorite and apochromatic glass. Still other objectives are capable of wavelength transmission of 400-1200nm by using proprietary coating, glass elements, and cementing agents [2].

The use of various devices that interfere with the light path constructively by enhancing contrast has continued to increase in usage in the last several decades. Specially modified objectives like phase, Nomarski, Hoffman and polarized light are necessary for these techniques to even happen. Light sources (sometimes used with interference filters and dichroic mirrors) for image enhancement such as mercury, xenon, IR, tin halide, and lasers are also expanding the use of specialty objectives.

References

- [1] M. Abramowitz, Microscope: Basics and Beyond, Olympus America, Inc., Melville, NY, (1987)
- [2] M. Abramowitz, & M. Davidson, Optical Microscopy, Olympus Microscopy Resource Center



Fig. 1.

Optical Correction in Objectives



Fig. 2. Levels of optical correction for aberration in commercial objectives.

(a) Achromatic objectives, the lowest level of correction, contain two doublets and a single front lens; (b) Fluorites or semiapochromatic objectives, a medium level of correction, contain three doublets, a meniscus lens, and a single front lens; and (c) Apochromatic objectives, the highest level of correction, contain a triplet, two doublets, a meniscus lens, and a single hemispherical front lens.

	Objective Type					
	Plan Achromat		Plan Fluorite		Plan Apochromat	
Magnification	N.A	Resolution (µm)	N.A	Resolution (µm)	N.A	Resolution (µm)
4x	0.10	2.75	0.13	2.12	0.20	1.375
10x	0.25	1.10	0.30	0.92	0.45	0.61
20x	0.40	0.69	0.50	0.55	0.75	0.37
40x	0.65	0.42	0.75	0.37	0.95	0.29
60x	0.75	0.37	0.85	0.32	0.95	0.29
100x	1.25	0.22	1.30	0.21	1.40	0.20
N.A. = Numerical Aperture						

Resolution and Numerical Aperture by Objective Type

Fig. 3.