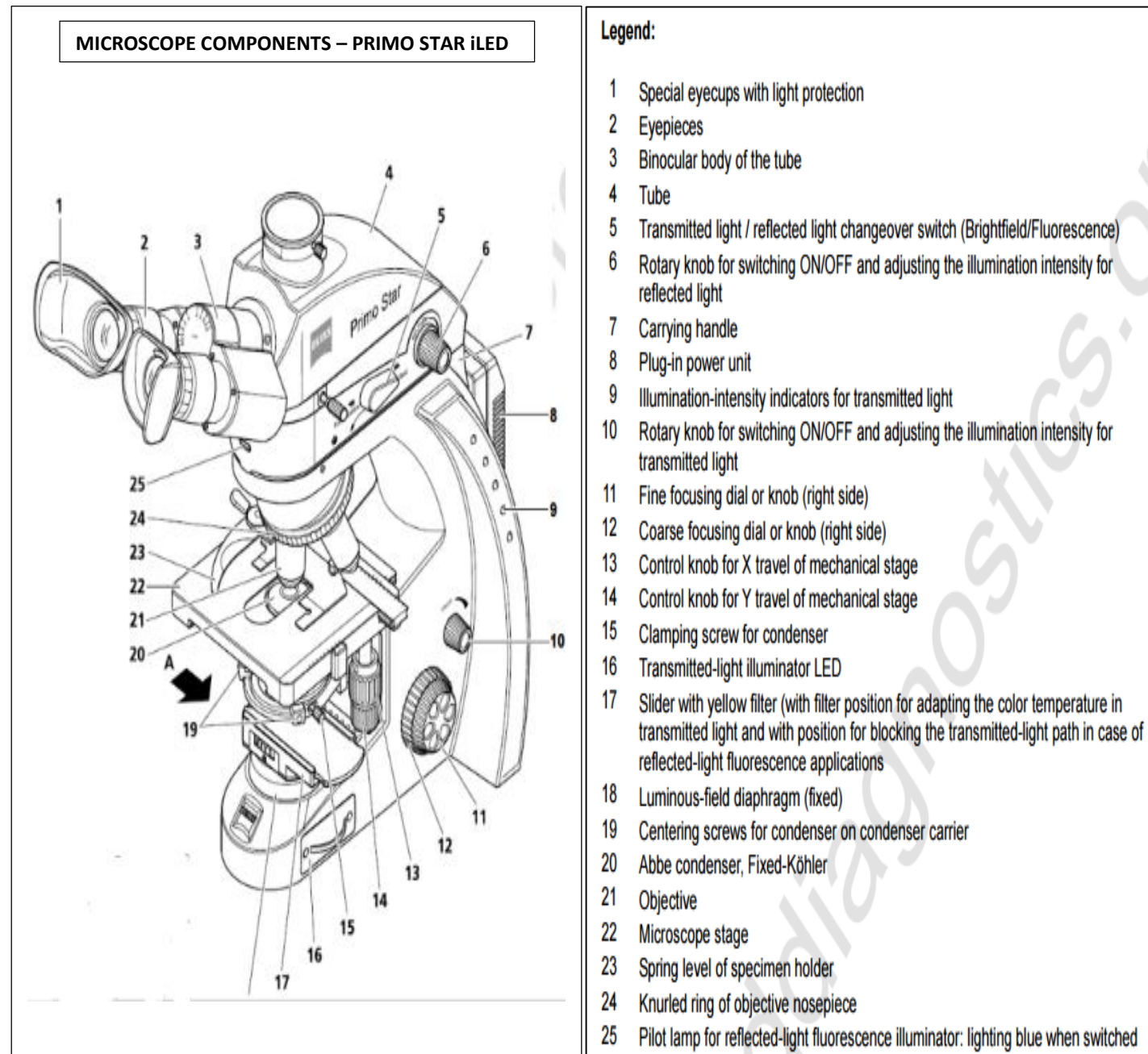


Grading of Smears in FM

IUATLD/WHO scale	FM 200x, 1 length = 30 fields	FM 400x, 1 length = 40-50 fields
Negative	No AFB in one length	No AFB in one length
*Confirmation Required	1-4 AFB in one length	1-2 AFB in one length
Exact Number (scanty)	5-49 AFB in one length	3-24 AFB in one length
1+	3-24 AFB in one field	1-6 AFB in one field
2+	25-250 AFB in one field	7-60 AFB in one field
3+	>250 AFB in one field	>60 AFB in one field



Trouble Shooting- Fluorescence Microscopy

Problem	Cause	Remedy
Too much fluorescence	Insufficient decolorization	Check decolorization time
	Counterstain too weak	Prepare new reagent
	Auramine has dried on the smear	Check smears are level over sink
		Add sufficient stain
	Auramine not filtered	Filter auramine at time of use
Pale acid-fast bacilli	Smear too thick	Prepare new smear
	Auramine has expired or stored in direct sunlight	Replace reagent
		Store bottle in the dark
	Auramine <0.1%	Recheck stain preparation and QC results
	Staining time <20 minutes	Stain for at least 20 minutes
	Smear overheated during fixation step	Pass smear through flame 3 times, 1-2 seconds each time
	Over decolourised	Do not exceed the maximum time (1-2 minutes only)
Background too dark	Stained smears exposed to daylight	Keep slides in the dark using slide box
		Read smears as soon as possible
	Smear too thick	Prepare new smear
	Counterstained too long	Do not exceed 1 minute
	Decolourised too long	Do not exceed 2 minutes
Background too dark	Inadequate washing step after counterstaining	Extend washing step
		Ensure washing step is complete
	Counterstain concentration too strong	Recheck stain preparation and QC results
	Smear too thick	Prepare new smear